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SUMMARY TECHNICAL REPORT
OF THE
NATIONAL DEFENSE RESEARCH COMMITTEE

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Manuscript and illustrations for this volume were prepared for publication by the Summary Reports Group of the Columbia University Division of War Research under contract OEMsr-1131 with the Office of Scientific Research and Development. This volume was printed and bound by the Columbia University Press.

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This volume, like the seventy others of the Summary Technical Report of NDRC, has been written, edited, and printed under great pressure. Inevitably there are errors which have slipped past Division readers and proofreaders. There may be errors of fact not known at time of printing. The author has not been able to follow through his writing to the final page proof.

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SUMMARY TECHNICAL REPORT OF DIVISION 9, NDRC

VOLUME 1

CHEMICAL WARFARE AGENTS, AND RELATED CHEMICAL PROBLEMS

[v.1]

Parts I-II

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WASHINGTON, D. C., 1946



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CHANGES ON THE ORGANIZATION OF NDRC

The duties of the National Defense Research Committee were (1) to recommend to the Director of OSRD suitable projects and research programs on the instrumentalities of warfare, together with contract facilities for carrying out these projects and programs, and (2) to administer the technical and scientific work of the contracts. More specifically, NDRC functioned by initiating research projects on requests from the Army or the Navy, or on requests from an allied government transmitted through the Liaison Office of OSRD, or on its own considered initiative as a result of the experience of its members. Proposals prepared by the Division, Panel, or Committee for research contracts for performance of the work involved in such projects were first reviewed by NDRC, and if approved, recommended to the Director of OSRD. Upon approval of a proposal by the Director, a contract permitting maximum flexibility of scientific effort was arranged. The business aspects of the contract, including such matters as materials, clearances, vouchers, patents, priorities, legal matters, and administration of patent matters were handled by the Executive Secretary of OSRD.

Originally NDRC administered its work through five divisions, each headed by one of the NDRC members. These were:

Division A — Armor and Ordnance

Division B — Bombs, Fuels, Gases, & Chemical Problems

Division C — Communication and Transportation

Division D — Detection, Controls, and Instruments

Division E — Patents and Inventions

In a reorganization in the fall of 1942, twenty-three administrative divisions, panels, or committees were created, each with a chief selected on the basis of his outstanding work in the particular field. The NDRC members then became a reviewing and advisory group to the Director of OSRD. The final organization was as follows:

Division 1 — Ballistic Research

Division 2 — Effects of Impact and Explosion

Division 3 — Rocket Ordnance

Division 4 — Ordnance Accessories

Division 5 — New Missiles

Division 6 — Sub-Surface Warfare

Division 7 — Fire Control

Division 8 — Explosives

Division 9 — Chemistry

Division 10 — Absorbents and Aerosols

Division 11 — Chemical Engineering

Division 12 — Transportation

Division 13 — Electrical Communication

Division 14 — Radar

Division 15 — Radio Coordination

Division 16 — Optics and Camouflage

Division 17 — Physics

Division 18 — War Metallurgy

Division 19 — Miscellaneous

Applied Mathematics Panel

Applied Psychology Panel

Committee on Propagation

Tropical Deterioration Administrative Committee

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NDRC FOREWORD

AS EVENTS of the years preceding 1940 revealed more and more clearly the seriousness of the world situation, many scientists in this country came to realize the need of organizing scientific research for service in a national emergency. Recommendations which they made to the White House were given careful and sympathetic attention, and as a result the National Defense Research Committee (NDRC) was formed by Executive Order of the President in the summer of 1940. The members of NDRC, appointed by the President, were instructed to supplement the work of the Army and the Navy in the development of the instrumentalities of war. A year later, upon the establishment of the Office of Scientific Research and Development (OSRD), NDRC became one of its units.

The Summary Technical Report of NDRC is a conscientious effort on the part of NDRC to summarize its work and to present it in a useful and permanent form. It comprises some seventy volumes broken into groups corresponding to the NDRC Divisions, Panels, and Committees.

The Summary Technical Report of each Division, Panel, or Committee is an integral survey of the work of that group. The first volume of each group's report contains a summary of the report, stating the problems presented and the philosophy of attacking them, and summarizing the results of the research, development, and training activities undertaken. Some volumes may be "state of the art" treatises covering subjects to which various research groups have contributed information. Others may contain descriptions of devices developed in the laboratories. A master index of all these divisional, panel, and committee reports which together constitute the Summary Technical Report of NDRC is contained in a separate volume, which also includes the index of a microfilm record of pertinent technical laboratory reports and reference material.

Some of the NDRC-sponsored researches which had been declassified by the end of 1945 were of sufficient popular interest that it was found desirable to report them in the form of monographs, such as the series on radar by Division 14 and the monograph on sampling inspection by the Applied Mathematics Panel. Since the material treated in them is not duplicated in the Summary Technical Report of NDRC, the monographs are an important part of the story of these aspects of NDRC research.

In contrast to the information on radar, which is of widespread interest and much of which is released to the public, the research on subsurface warfare is largely classified and is of general interest to a more restricted group. As a consequence, the report of Division 6 is found almost entirely in its Summary Technical Report, which runs to over 20 volumes. The extent of the work of a division cannot therefore be judged solely by the number of volumes devoted to it in the Summary Technical Report of NDRC: account must be taken of the monographs and available reports published elsewhere.

Under the leadership of Walter R. Kirner as Chief, Division 9 conducted a broad program of research in the field of chemical warfare, both for offense and defense. Its principal responsibility was to ensure that this country would be prepared, should the enemy resort to the employment of poison gas as an offensive weapon.

The staff of the Division prepared some two thousand chemical compounds, and tested them for toxicity and vesicancy at a central laboratory. During the course of this program, a number of new chemical warfare agents were discovered which were potential deadly weapons. For defense, the Division contributed to the development of methods and equipment for detecting and protecting against chemical agents, in vapor form or dissolved in water; important work was done in the development of an improved type of impregnated clothing. The Division also worked with the Committee on Medical Research in the problem of new anti-malarial agents, insecticides, and rodenticides.

The Summary Technical Report of Division 9, prepared under the direction of the Division Chief and authorized by him for publication, is a record of this work, a great deal of which constituted an insurance policy against a threat which did not materialize. We can be thankful therefore, and we are grateful to the staff of Division 9 for its vital contributions in the field of chemical research.

VANNEVAR BUSH, Director
Office of Scientific Research and Development

J. B. CONANT, Chairman
National Defense Research Committee

FOREWORD

DIVISION 9, also known as the Chemistry Division, specialized mainly in chemical warfare problems. Its activities were concerned with problems of both offense and defense. A large part of the program involved a search for new candidate chemical warfare agents particularly of the so-called persistent types. Nearly two thousand of such compounds were prepared. The most promising candidates were carried through the pilot plant so as to secure engineering data on their preparation and also to provide sufficient material for further evaluation in the laboratory and in the field. The Division maintained a large central laboratory in which the candidate compounds were screened for toxicity and vesicancy. As a result of this program a number of new chemical warfare agents were discovered possessing the necessary toxicity and other desirable properties. In addition, certain improvements were suggested for the synthesis of some of the agents which had previously been standardized for chemical warfare use.

On the defensive side, Division 9 made important contributions to the development of methods and equipment for the detection and analysis of chemical warfare agents in the vapor form or dissolved in water. Procedures were also devised for the removal of such agents from water. A great deal of effort was expended in the development of protective clothing. Division 9 investigators discovered stabilizers for the chemically-impregnated clothing manufactured by the Chemical Warfare Service, which greatly extended its storage life. A new, so-called "aqueous impregnation process" for protective clothing was developed which avoided shipment of large quantities of organic solvents to war theatres. Several kits were devised with which an individual or group of soldiers could impregnate their clothing in the event of an emergency.

An intensive search was made for substitute impregnates to replace the one adopted by the Army. Certain of these new compounds, particularly several first prepared by Naval Research Laboratory investigators, proved of outstanding value for use in protective ointments. These agents were incorporated into the protective ointments standardized, manufactured, and distributed by the Army and Navy to personnel throughout the world, after their efficacy for this purpose had been discovered by Division 9 investigators and methods developed for improving

their synthesis and compounding them into ointments.

A new approach to the problem of protective clothing was undertaken by Division 9 in its work on carbon-impregnated clothing following a lead furnished by the British. Three different methods of impregnating carbon into cloth were successfully developed, two of which show unusual promise. The advantage possessed by carbon-impregnated fabrics over chemically-impregnated fabrics is that the former will protect the wearer against all known persistent agents whereas the latter is limited to protection against agents of the mustard-gas type.

Division 9 also carried on an extensive research program on the physiological mechanism of action of chemical warfare agents. The goal of this program was the discovery of effective methods of therapy to be used against gases which might be used by the enemy. While this did not result successfully in the case of mustard gas, a much clearer understanding was reached as to the mechanism by which this agent produces cell injury and vesication. The discovery of BAL by the British has made available a very effective antidote against vesication by arsenical war gases of the Lewisite type. Toward the end of the war, when the effectiveness of the flame thrower was demonstrated in attacks on Japanese entrenched in caves and pill boxes, this program was extended to include a basic study of the physiological mechanism of action of heat on animals.

Division 9 personnel participated in the field evaluation of chemical warfare agents at all of the Chemical Warfare Service proving grounds. Practically all of the analytical work done at the Dugway Proving Ground Mobile Field Unit Installation, Bushnell, Florida, was performed by Division 9 men on loan from its various contractors. Much of the analytical equipment used in these field tests was developed by Division 9 investigators. The important discovery made during these experiments under sub-tropical conditions was the considerably enhanced activity of mustard gas at high temperatures and high humidity.

When it became evident that chemical warfare would not be initiated by the enemy or by the Allies, Division 9 shifted its emphasis from chemical warfare problems to other urgent chemical problems. It cooperated with the Committee on Medical Research

in a search for new, effective anti-malarial agents, insecticide formulations, insect repellents, and rodenticides. The discovery of the new rodenticide "1080" was made jointly by investigators of Division 9 and the Fish and Wildlife Service, Department of the Interior.

Division 9, NDRC, was created in December 1942, at the time the Office of Scientific Research and Development was organized and the NDRC reorganized. Its predecessor Sections in Division B were first Sections A-2, A-3, and A-4 and later Sections B-3 and B-4. The early organization of the scientific work of these Sections had been most effectively carried out by Drs. Roger Adams, H. S. Gasser, W. C. Johnson, and C. S. Marvel. Their leadership was one of the important factors which contributed to the successful solution of many of the problems assigned to Division 9 by the Army and Navy. The other important factor was the ability, industry, and enthusiasm of the official investigators and their associates and assistants in university and industrial laboratories in attacking the problems which were, in turn, assigned to them. Generous credit should also be given to the Division Members and Technical Aides who carried out the scientific administration of the many contracts which were under the aegis of Division 9. It is a pleasure to gratefully acknowledge here the assistance loyally rendered by all of these men in the laboratory accomplishments described in

detail in the Division 9 Summary Technical Report, presented herewith.

Particular expressions of gratitude are due the authors of the chapters which constitute the volumes summarizing the work of Division 9. Because of the policy adopted by this Division to summarize critically not only the work of its own investigators, but also the contemporary work done in American Service laboratories and in the laboratories of our Allies, the task of writing was made considerably more difficult. However, it is believed that this overall summary will greatly add to the value of the volumes by giving as complete a picture as possible of present knowledge on each subject.

Finally, special acknowledgment is made of the outstanding work done by Dr. Birdsey Renshaw, the editor of the Division 9 Summary Technical Report. He organized the report, coordinated the efforts of the authors of the respective chapters, wrote all or part of a considerable number of the chapters, and carefully edited each chapter as it was completed. This work has required his full time attention for well over a year during which his own desires to carry on laboratory work had to be postponed. However, now that the task is completed he will, I am sure, derive a great deal of satisfaction from having done it so well.

W. R. KIRNER
Chief, Division 9

PREFACE

IT WAS THE CONSENSUS in Division 9 that the value of its Summary Technical Report, requested as a supplement to the numerous detailed reports already prepared and issued, would be greatly enhanced if an attempt were made not only to summarize the Division's work but, in addition, to review critically the information available from other sources and relating to the subjects on which the Division had undertaken investigations. This seemed particularly desirable because in most phases of the work the contributions of the Division and those of other agencies in the United States and British Commonwealth of Nations supplemented and reciprocally influenced each other. Furthermore, the data on most of the pertinent subjects are scattered in numerous reports of various origins which in the future will be difficult to locate and evaluate. It seemed worthwhile, therefore, also to include a fairly complete Bibliography.

This undertaking has been pursued, for the most part subsequent to the defeat of Japan, by men who had actively participated in the work of the Division. Most of the authors were burdened with other duties and have made considerable personal sacrifices to write the summaries. Nevertheless, they have attempted conscientiously to present in useful form the significant facts and concepts that emerged relative to their subjects during World War II. In so far as this aim has been attained, the reader will no

doubt be willing to overlook stylistic and editorial heterogeneities. Unfortunately, it is inevitable that occasional omissions and errors must creep into a rapid compilation and assessment of as much material as is included in this Report. These the reader will accept with less equanimity, and for them the authors and the Editor ask forgiveness.

By the time the Division closed in 1946 practically all of its work had been presented in detail in OSRD Formal Reports. These, therefore, comprise most of the references to the Division's work that are given in the chapters and reproduced in microfilm. Most of the numerous informal and miscellaneous reports issued during the war were of an interim nature. They have been referred to and microfilmed only when they appeared to possess permanent value or included material not incorporated in OSRD Formal Reports. Among the informal reports that may appropriately be made a part of the accessible permanent record are the Section 9:4:1 (formerly B4-A) Informal Reports on *Toxicity of Chemical Warfare Agents*, and the Section 9:5:1 (formerly B6-C) Informal Reports on *Physiological Mechanisms of Chemical Warfare Agents*. Both of these series are included *in toto* in microfilm.

BIRDSEY RENSHAW
Editor

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^a For facility of handling, this Summary Technical Report of Division 9, Volume 1, has been bound and published in two sections. The first section contains Part I and Part II of Volume 9-1. Parts III-VI are found in the second section, together with the Glossary, Bibliography, OSRD Appointees, Contract Numbers, Project Numbers, and Index, all of which are applicable to both sections of Volume 9-1.

PART I

***PREPARATION AND EVALUATION OF POTENTIAL
CHEMICAL WARFARE AGENTS***

Chapter 1

RÉSUMÉ OF AGENT ASSESSMENTS

By *Stanford Moore* and *Birdsey Renshaw*

1.1 INTRODUCTION

REVIEWS OF THE information on the principal standard and potential chemical warfare agents are presented in Chapters 2 through 14. Summaries of the data on the standard agents — mustard gas, phosgene, hydrogen cyanide, and cyanogen chloride — are accompanied by reviews of other potential chemical warfare agents that were investigated during World War II. The reviews and bibliographies are not limited to data obtained by the National Defense Research Committee [NDRC]. So far as possible all available information has been considered.

The chapters deal primarily with the laboratory data on the chemical and toxicological properties of the agents. The technical aspects of the use of chemical warfare agents in the field have recently been summarized by the Project Coordination Staff.¹

In the discussions presented in this volume it is assumed that under certain conditions the use of each of the standard agents would be more effective from a military point of view than a similar expenditure of munitions charged with high explosive.² The experimental agents are assessed relative to the standard chemical agents on this basis. *It has not been possible to take into consideration the recent development of the atomic bomb as a high-explosive weapon, the highly toxic radioactive gases encountered in the course of the research leading to the production of the atomic bomb, or the military potentialities of bacteriological warfare. For this reason the assessments made in the following chapters are limited in scope and cannot be considered complete in the broadest sense.*

1.2 MAJOR TRENDS SINCE WORLD WAR I

MUSTARD GAS VAPOR

From the laboratory and field test data obtained by the United States and the United Kingdom there developed during World War II a growing realization of the effectiveness of mustard gas vapor as a potential offensive weapon, particularly in tropical climates, in addition to its well-defined role as a de-

fensive weapon. Emphasis in the field testing was placed on the thorough assessment of vapor dosages, as well as on the contact hazard presented by the contamination of various types of terrain with the liquid agent. It was demonstrated¹ that in hot weather relatively moderate expenditures of munitions would yield severely incapacitating vapor dosages within less than an hour, although the time of onset of the incapacitating symptoms was from 12 to 24 hours after the brief exposure period. The effects were optimal on heavily vegetated tropical terrain of the type characteristic of some of the combat areas in the war against Japan.

ARSENICAL VESICANTS

At the close of World War I lewisite gained almost legendary fame as a potential vesicant agent. More recently thorough assessment of the arsenical vesicants has shown lewisite to possess few if any advantages over mustard gas and to have several properties which greatly reduce its efficiency. Its marked susceptibility to hydrolysis, for example, lowers the vapor return from contaminated terrain and decreases the effectiveness of the vesicant through clothing. The development of British anti-lewisite [BAL] as an antidote in arsenical poisoning also affected the assessment. Lewisite and related arsenical vesicants have, of recent date, received little consideration in the United States as potential offensive agents.

STERNUTATORS AND LACRIMATORS

Interest in sternutators and lacrimators has markedly decreased since World War I. Particulate filters effective against sternutators are now standard equipment in the gas masks of all countries. In so far as harassment by chemical agents is a legitimate military objective, there is a tendency to prefer the use of agents that are potentially lethal, particularly since British field trials under simulated combat conditions have demonstrated that the effectiveness of sternutators as harassing agents is limited.

USE OF NONPERSISTENT AGENTS

As a means of attaining casualties by use of non-persistent gas against troops equipped with masks,

attention has been focused on surprise attacks with large bombs and on the possibilities of "breaking" the canister with high gas concentrations. Under favorable conditions of terrain and meteorology it has been found possible to attain with cyanogen chloride dosages that effectively penetrate the World War II models of German and Japanese canisters. The currently available United States canisters filled with ASC charcoal give effective protection against several times these dosages.

HIGH EXPLOSIVE-CHEMICAL MUNITIONS

German developments at the close of World War I pointed to the potentialities of high explosive-chemical shell. In 1921 Fries and West³ noted with regard to mustard gas that:

Due to the very slight concentrations ordinarily encountered in the field, resulting from a very slow rate of evaporation, the death rate is very low, probably under 1 per cent among the Americans gassed with mustard during the war.

If, on the other hand, the gas be widely and very finely dispersed by a heavy charge of explosive in the shell, the gas is very deadly. In such cases the injured breathe in minute particles of the liquid and thus get hundreds of times the amount of gas that would be inhaled as vapor. This so-called "high explosive mustard gas shell" was a German development in the very last months of the war. Its effects were great enough to make it certain that in the future large numbers of these shell will be used.

The Germans followed this lead in their developmental work during World War II. They apparently favored chargings that could be dispersed from munitions not readily distinguishable by sound from similar high-explosive weapons and that would give relatively odorless vapor and droplet clouds. For this purpose the Germans filled shell and bombs with the nitrogen mustard, *tris*(β -chloroethyl)amine (HN3), and with the so-called Trilons (see Chapters 6 and 9). From the available data it would appear that the use of munitions of this type in admixture with high explosive munitions would have been an effective means of employing chemical agents. A similar employment of 4.2-inch mortar shell charged with a nitrogen mustard was considered by the United States Chemical Warfare Service and would have received further attention in the event of the outbreak of gas warfare. It would appear that the Germans placed greater emphasis on this method of using gas and had obtained more data than the United States and the United Kingdom on the properties of high explosive-chemical shell and on the relative suitabilities of various chargings for these munitions. Field assessments of the lethal effectiveness of particulate

clouds of ricin and of the clouds obtained from incendiary weapons containing cadmium were obtained by the United States and the United Kingdom and also bear on this problem. It is probable that future research in the United States will augment the data on the Trilons and on the effectiveness of particulate clouds of high toxicity.

TOXICOLOGICAL TESTING

There is a general realization of the need for continued toxicological screening on a broad basis of newly synthesized organic chemicals. The German Trilons were synthesized in the course of a search by industry for new insecticides. Routine testing on animals brought them immediately into consideration for military uses. For a routine survey program the use of intravenous injections is simpler than determination of toxicity by inhalation and suffices to detect compounds which merit further study.

Experience gained during World War II has emphasized the importance of obtaining an approximation of the toxicity of agents for the human species. This point becomes evident from the studies on disulfur decafluoride, the fluorophosphates, the fluoroacetates, and cadmium oxide. The various animal species showed such widely varying susceptibilities to the agents that estimates of the order of magnitude of their effectiveness against man could not be made without collateral data. The fundamental physiological and biochemical studies leading to a closer definition of the lethal dose of hydrogen cyanide for man (see Chapter 2) provide another example. Thus the possibility of wide species variations merits consideration at the outset of detailed study. In screening by intravenous injection, the use of several species at an early stage is essential to the adequate assessment of the potentialities of a new compound.

1.3 BRIEF SUMMARY OF ASSESSMENTS OF COMPOUNDS OF PRINCIPAL INTEREST

HYDROGEN CYANIDE AND CYANOGEN CHLORIDE

For the task of production of immediate deaths by the attainment of effective dosages in less time than is required for masking, hydrogen cyanide (AC) was considered from the toxicological point of view to be the best available agent. Its competitors which arose during World War II are the almost equally quick-acting but much less volatile fluorophosphates and related compounds, one of which the Germans de-

cided to put into production for use in high explosive-chemical shell and bombs. The tendency of AC to flash in some munitions is a disadvantage which has not been overcome.

In the United States cyanogen chloride (CK) was second choice to AC in the task cited in the preceding paragraph and was also considered for the special task of penetrating early World War II models of Japanese and German canisters under highly favorable conditions of terrain and meteorology.

PHOSGENE

Phosgene (CG) was the principal nonpersistent gas of World War I and was the standard nonpersistent chemical filling for United States bombs and mortar shell at the outbreak of World War II. When delayed physiological effects are acceptable, CG has been considered the most economical standard nonpersistent agent for the production of casualties by attainment of effective dosages in less time than is required to mask and for the production of casualties among unprotected personnel.

DISULFUR DECAFLUORIDE

The difficulty and expense of manufacture of disulfur decafluoride (Z) on a large scale have prevented it from being seriously considered as a competitor for CG. The agent possesses the important advantage of relative lack of odor, and its physical properties are satisfactory. However, its toxicity shows considerable species variation and the lethal dosage for man cannot at present be estimated with a degree of accuracy sufficient for a close toxicological comparison with CG.

SULFUR MUSTARDS

No competitors for *bis*(β -chloroethyl) sulfide (H) were developed in the course of the study of a wide variety of analogs after World War I and during World War II. The search for a more volatile (i.e., less persistent) vesicant agent led only to compounds with markedly inferior toxicological potency. The high vesicancy of some of the less volatile analogs was studied quantitatively, however, and found to be sufficient to merit the addition to H of 1,2-*bis*(β -chloroethylthio)ethane (Q) or *bis*(β -chloroethylthioethyl) ether (T) when long persistency of contact hazard on terrain or matériel is desired.

NITROGEN MUSTARDS

The principal item of military significance that

emerged from the study of the nitrogen mustards was the possible use of *tris*(β -chloroethyl)amine (HN3) as a filling for high explosive-chemical shell. For most other purposes where a vesicant agent could be employed to advantage H was shown to have properties superior to those of the nitrogen mustards.

ARSENICALS

The thorough assessment of the arsenical vesicants indicates that lewisite (L) and analogs are in general inferior to the sulfur mustards. Subsequent to the introduction of silver impregnation for canister fillings arsine has not seriously been considered as a potential agent. Relatively little military value is currently attached to the use of irritant arsenical smokes.

ALIPHATIC NITROSOCARBAMATES

Studies on methyl N-(β -chloroethyl)-N-nitrosocarbamate (KB-16) and related structures showed this series of compounds to possess a high degree of toxicity. As an eye-injuring KB-16 is approximately as potent as H and is less readily detected by odor. As a vesicant, however, it is markedly inferior to H. It has also been found to be insufficiently stable for storage in munitions.

FLUOROPHOSPHATES AND TRILONS

In 1941 British investigators initiated studies on the fluorophosphates as potential war gases. The most effective members of the series studied by the United States and the United Kingdom were dimethyl fluorophosphate (PF-1) and diisopropyl fluorophosphate (PF-3). PF-1 and PF-3 do not possess sufficiently high toxicities by inhalation to have an advantage over the standard chemical warfare agents. Limited data are available on the related agents (Trilons) discovered by the Germans during World War II. The member of the series which they placed in production was ethyl dimethylamidocyanophosphate (MCE). Difficulty in synthesis prevented the Germans from producing isopropyl methane-fluorophosphonate (MFI), which apparently possesses superior chemical and toxicological properties. It is clear that the Trilons are more toxic than PF-1 or PF-3. They are quick-killing agents possessing a powerful parasympathomimetic action. The Trilons would seem to be the one new group of chemical agents discovered during World War II that merit a position among the standard agents.

FLUOROACETATES AND RELATED COMPOUNDS

Reports that methyl fluoroacetate (MFA) is highly toxic were received from Polish investigators by the British in 1942. This class of compounds received careful chemical and toxicological study in the United Kingdom and the United States. The wide species variation in toxicity pointed to the need for a reasonably accurate estimate of the lethal dose for the human species before an assessment could be made. If these agents had proved to be as toxic for man as for some animal species they would have been in a class with the Trilons. However, data becoming available in 1944 made it possible to estimate that the toxicity for man is low and to conclude that MFA and related compounds do not possess the general utility of the currently standardized gases. They remain a subject of military concern because of their possible use as food or water poisons. In addition, as a by-product of the chemical warfare research, sodium fluoroacetate has been demonstrated to have many practical properties as a rodenticide.

CADMIUM OXIDE; METAL CARBONYLS

Cadmium is considered a promising material for addition to incendiary munitions if toxicity as well as fire is desired. The highly toxic cadmium oxide which results from the combustion of the incendiary mixes is odorless and relatively nonirritating.

Iron and nickel carbonyls have been considered as possible additions to flame thrower fuels if increased toxicity of the combustion products is an objective.

RICIN

Ricin (W) is a protein of very high toxicity. The absence of odor and the complexity of the detection problem in the field would render it more insidious than any standard United States or British chemical warfare agent. As a result of progress made during World War II on the preparation of ricin, it is potentially available in relatively large quantities. Improved munitions for its dispersal as a particulate cloud are required before the toxicity of the agent can be adequately utilized. Comparison of the effectiveness of the initial dust cloud of ricin with that of the initial cloud from the German Trilons would be indicated. The casualty-producing effects from exposure to ricin, however, are delayed, like those of phosgene poisoning, whereas incapacitation and death from the Trilons is produced relatively rapidly.

AROMATIC CARBAMATES

Research by the United Kingdom and the United States during World War II has made available in pilot plant quantities a series of crystalline aromatic carbamates possessing extremely high toxicity. By subcutaneous injection the most effective members of the series are comparable in potency with the German Trilons. For some purposes the fact that they are crystalline solids is advantageous. For dispersion by high explosive-chemical shell the Trilons have the advantage of being liquids. The possible utilization of aromatic carbamates may rest primarily on the availability of suitable munitions.

Chapter 2

HYDROGEN CYANIDE AND CYANOGEN CHLORIDE^a

By *Stanford Moore* and *Marshall Gates*

2.1 INTRODUCTION

A RELATIVELY large amount of open and classified literature has accumulated on the properties of hydrocyanic acid and cyanogen chloride and on their behavior when liberated from munitions. To survey the complete subject is beyond the scope of this chapter. The sections which follow are designed to show the relationship of the data obtained by NDRC Division 9 to the body of knowledge on these agents.

Hydrocyanic acid (AC) was adopted by the United States as a standard filling for frangible grenades in July 1942 and as a substitute standard filling for 1,000-lb bombs in October 1943. From the toxicological standpoint this agent was one of the standards for comparison in the evaluation of potential new gas warfare agents under investigation by NDRC during World War II.

Cyanogen chloride (CK) was standardized as a quick-acting nonpersistent gas filling for 1,000-lb and 500-lb bombs in October 1943 and for 4.2-inch mortar shell in July 1945.

For the task of production of immediate deaths by the attainment of effective dosages in less time than is required for masking, AC is considered from the toxicological standpoint to be the best agent available.^{41,43} Its competitors are the almost equally quick-acting but much less volatile fluorophosphates and related compounds, one of which was put into production by Germany (Chapter 9). The tendency of AC to flash in some standard munitions is a disadvantage which has not been overcome.

The primary interest in CK arose from the demonstration by NDRC Division 10 that CK showed greater promise for the penetration of World War II models of Japanese and German canisters than any other readily available agent. The protection afforded by Japanese canisters in particular was so low that there was promise of attaining lethal penetration of enemy masks from moderate expenditures on jungle terrain or heavily wooded areas. CK can be satisfactorily stabilized for storage and is non-

inflammable. It is also second choice to AC in the task cited in the preceding paragraph.

In this chapter reference will be made to the primary chemical and toxicological basis on which the standardization of these agents rested.

2.2 PREPARATION AND PROPERTIES

2.2.1 Preparation

Hydrocyanic acid (AC) has long been available on a commercial scale. The cost of production at the present time is about five times that for phosgene. Cyanogen chloride (CK) was produced by the French in World War I and on a small scale in the United States prior to World War II. Larger scale production, by a process based upon the chlorination of aqueous hydrocyanic acid, was undertaken in 1944 at the plant in Azusa, California.

2.2.2 Physical Properties

The physical properties which have the most direct bearing on the effectiveness of these agents as war gases are the following:

	AC	CK
Density (liquid) g/ml at 20 C	0.69	1.19
Boiling point, C	25.7	12.6
Freezing point, C	-13.4	-7.0
Heat of vaporization, cal/g	210	135
Vapor density, relative to air	0.93	2.0

The liquid density affects the amount of agent which can be loaded per munition; the freezing point is of importance in cold weather or on high-altitude bombing missions; the boiling point influences the rate of evaporation of the liquid agent; the heat of vaporization also influences the rate of evaporation and determines the cooling of the air produced by evaporation of the agent from a functioned munition. The cooling effect in turn influences the stability of the gas cloud produced, since the cooler layer of air tends to remain longer near ground level. Prior to the performance of adequate field studies on these agents it was considered that, since AC has a lower vapor density than that of air, the persistence of its clouds should be much less than the persistence of those

^a Based on information available to Division 9 of the National Defense Research Committee [NDRC] as of November 1, 1945.

from CK and CG, which are heavier than air. Actually, clouds of AC and CK show essentially the same persistence and downwind travel.⁴¹ Considered in conjunction with the cooling effect arising from the latent heat of vaporization and with the meteorological factors governing the turbulence of the air, the data show that the vapor density of the gas does not play so important a role as was first thought.

The boiling points of AC and CK are sufficiently low so that the agents vaporize completely within a few seconds after dispersal in droplet form from bursting munitions.

2.2.3 Stability of AC

Pure AC is unstable on storage, ultimately decomposing with explosive violence, but the presence of small amounts of mineral acids, particularly phosphoric acid, produces marked stabilization. Present Chemical Warfare Service specifications call for the addition of 0.07 per cent orthophosphoric acid and 0.3 per cent sulfur dioxide as stabilizers, and surveillance tests have demonstrated that AC stabilized according to these specifications and charged into clean bombs is stable when held at 150 F for 60 to 90 days.^{20,35,41} Powdered copper, used by the Japanese as a stabilizer in AC munitions, has also shown promise as a stabilizer, particularly in the presence of steel, which gradually exhausts the phosphoric acid stabilizer.^{5k,n,o,r}

The tendency of AC to inflame on functioning of munitions constitutes the principal disadvantage of AC as a chemical warfare agent. In field trials with 500-lb and 1,000-lb bombs an appreciable percentage of the munitions flashed in some instances. Extensive efforts have been made to overcome or minimize this tendency both by changing the bursters and by altering the composition of the charging. The addition of aliphatic hydrocarbons in C₅-C₆ range originally appeared promising^{5c,g,j,k,n} and led to field tests on them and also on gasoline as flash-inhibiting diluents. The early trials indicated⁴⁰ that flashing could be reduced by the addition of 5 per cent of 70-octane gasoline to AC in M47A2 100-lb bombs, and in larger bombs, but subsequent work indicated that the addition of gasoline to AC does not satisfactorily prevent flashing if the mixture is allowed to stand for 20 days or longer after the addition of the gasoline.^{21d} No solution of the problem of flashing of bombs charged with AC which would keep the per cent flashing consistently below 10 per cent, for ex-

ample, has been obtained. The fact that in some series of trials no flashing is encountered indicates that the problem may not be an insoluble one.

2.2.4 Stability of CK

Pure CK, and also the CK produced technically in this country, may be kept in glass for long periods even at elevated temperatures.^{1,4,50,54} Stability becomes a problem only on storage in metals. Investigations have proceeded along three lines: (1) the determination of the effect of storage in contact with metals, particularly steel, on the stability of CK, (2) determination of the effects of the impurities encountered in technical CK on its storage life in steel, and (3) minimization of the effects of contact with metal and of impurities by the addition of a stabilizer.

The chemical change involved in the polymerization of CK is largely one of trimerization to cyanuric chloride^{54,58,59} but other reactions, the products of which were undetected until recently, also occur to a minor extent.⁵ It was recognized many years ago^{51,54} that acids, particularly hydrochloric acid, promote the polymerization to a marked degree. The addition of 2 per cent of hydrochloric acid is sufficient to produce explosive polymerization,¹⁷ but the amount of hydrochloric acid likely to be present in technical CK is harmful only if appreciable water is also present.^{51,8f} The presence of chlorine and hydrogen cyanide were also held to be deleterious by early workers.^{58,59} Chlorine is still regarded as harmful,^{5a,47} although there is some evidence that it is less critical than formerly supposed. However, it presents no problem since it is well controlled in the manufacturing process. The amount of AC present can be varied within wide limits without affecting the stability of CK.^{4,5a}

Work on the stabilization of CK was undertaken in 1942 by NDRC Division 10. The early work was confined to experiments on pure CK stored in glass at room temperatures.¹ The harmful effects of chlorine and gross quantities of acid were observed, and it was noted that cyanuric chloride had no effect on the rate of polymerization. Later work, however, has shown that this is true only in the absence of steel^{8a,19} and also that water, observed in this early work to have little effect even in gross quantities, is actually very harmful to CK stored in steel.⁴ Magnesium oxide effectively stabilizes CK against the harmful effect of chlorine. Canadian workers have observed that magnesium oxide also protects against excess acidity⁴⁸ but it does not appear to meet the

requirements for a good stabilizer.⁴⁷ A number of other substances were examined for their effects on the stability of CK, but the surveillance tests, done at room temperature over relatively short periods (i.e., 2 to 6 weeks), were not sufficiently rigorous to give information useful in predicting the stability of munitions charged CK.

In 1943 the study of the stabilization of CK was intensified by NDRC Division 10, and much fundamental chemistry relating to CK, in particular to those reactions taking place during the storage of crude CK, was elucidated.

A detailed description of this work is given elsewhere.^b The more important results are enumerated here.

1. Although the earlier work showing the general deleterious effect of acids and of chlorine on the stability of CK was confirmed,^{5a} the special position of water, particularly in the presence of steel, was recognized.^{5a,1} Thus the stability of CK in the presence of steel decreases with increasing original water content, whereas no such correlation is shown with acid or with hydrogen cyanide.^{38a} Hydrogen chloride, AC, and ammonium chloride all cause little polymerization if moisture is absent.^{5a,1}

2. A number of stabilizers were proposed and tested. They were suggested before the importance of iron salts and of water was realized, and the rationale for most of them was removal of acid. Propylene oxide, first proposed as a stabilizer by the American Cyanamid Corporation,¹⁷ and ethylene oxide absorb acid even when dry^{5d} and were at first believed to be promising stabilizers. Later work showed them to be definitely harmful.⁴ Dimethylcyanamide, an end product of the reaction of CK with trimethylamine, a reaction analogous to von Braun's "bromcyan" degradation, absorbs two moles of acid.^{5g} It was suggested for trial as a stabilizer by the fact that no polymerization occurs during its formation from CK and trimethylamine.^{5f} However, the stabilizing effect appears to be due primarily to the production of a coating on the steel, and, if the sample is agitated during surveillance, no stabilization results.⁴ Likewise, it was possible to demonstrate a stabilizing effect due to the formation of the complex $(\text{HCN})_2 \cdot (\text{HCl})_3$ only if a considerable excess of AC was present, and then the stabilization was slight.^{5e}

Parallel to this work and supplementing it, the Chemical Warfare Service carried on surveillance

tests in munitions charged CK, originally on CK produced by the American Cyanamid Corporation at Warners, New Jersey, and later on CK produced at Azusa, California, as well. The results of these surveillance tests, which were carried out for 30, 60, and 90 days and ultimately for longer periods at 65 C, in general confirmed the conclusions reached in laboratory scale tests as to the effects produced by the usual impurities in technical CK. Good quality CK was shown to have adequate stability when charged into clean munitions.^{17,18,19} These surveillance tests also afforded realistic opportunities for the evaluation of stabilizers developed in laboratory scale tests.

The NDRC Division 9 group which undertook a search for stabilizers for CK during 1944 observed the beneficial effects of a number of inorganic substances including sodium pyrophosphate, calcium oxide, and potassium fluoride. In the interest of expediency it proved necessary to carry out surveillance tests on these stabilizers at 100 C and 125 C.^{8c} The applicability of the results of such accelerated tests to stability at lower temperatures was a matter of detailed study. All groups engaged in laboratory scale surveillance tests on CK ultimately adopted these temperatures. The rate of polymerization of CK increases by the usual factor of 2 per 10-degree rise in temperature (determinations have given 1.7 to 2.5) and the character of the polymerization appears to be the same at 125 C as at 75 C.^{8b} However, ammonium chloride in CK produces acid in surveillance at 100 C but not at 65 C,^{12b} and it is possible that surveillance at higher temperatures may lead to an underestimate of stability at lower temperatures.^{12a,c,f}

The effectiveness of sodium pyrophosphate, the best of the above-mentioned group, and of calcium oxide is much greater than that of organic stabilizers such as ethylene and propylene oxides and the dialkylcyanamides, which in spite of early promise were shown to be definitely deleterious.^{8a} Marked improvement in the storage qualities of even poor grade CK is obtained with sodium pyrophosphate. For CK of average quality, 2 per cent sodium pyrophosphate is adequate, although 5 per cent has been recommended and adopted to make certain that poor batches receive adequate stabilization.⁴ With this concentration of stabilizer, CK of any grade has better keeping qualities in the presence of steel than has the same CK stored in glass but unstabilized.⁴ Poor quality CK has a short life even in glass, how-

^b See the Summary Technical Report of Division 10.

TABLE 1. Surveillance of CK (Azusa Lot 686) for 60 days at 65 C.²²

Munition	Na ₂ P ₂ O ₇ (%)	Density	Acidity as HCl	Soluble residue	Iron	H ₂ O	HCN
M78 bomb	0			Polymerized after 60 days			
M78 bomb	5	1.204	0.017	0.06	0.001	0.051	1.94

ever, and to be suitable for charging into munitions CK should have a solidification time in glass at 125 C of at least 10 days.^{4,8c,f,g} This implies low values for soluble iron and for water content.

An example of the behavior of actual munitions charged CK when stabilized with sodium pyrophosphate is given in Table 1.²²

The stability of CK samples which have already been stored in steel without a stabilizer is markedly improved by the addition of 5 per cent sodium pyrophosphate, and CK thus treated has at least as long a life in steel as when stored in glass without a stabilizer.^{8g}

There appears to be some correlation between the stability of a sample of CK and its soluble iron content.^{8g} A corresponding correlation between water content and stability appears at low values of water content^{8g,12a} but is much less marked for higher values, the critical value being about 0.2 per cent water.^{8e} However, the susceptibility to stabilization by sodium pyrophosphate is strikingly dependent on water content, as illustrated by the data of Table 2.⁴

Although only limited data are available, the presence in CK of potassium pyrophosphate and presumably of sodium pyrophosphate appears to have little effect on its content of water, iron, acid, or hydrogen cyanide, although the rate of trimer formation is reduced. Over a 9-day period less than 0.002 per cent potassium pyrophosphate dissolves in CK.⁷

Since the stabilizing action of sodium pyrophosphate is probably a surface phenomenon the particle size is important. "Kiln run" or "granular" sodium

pyrophosphate is unsuitable, and it is necessary to include in the specifications a clause, easily met by the commercial "powder" grade, specifying the proper screening characteristics. A suitable set of specifications, readily met by commercial material, has been suggested.^{8e}

Little or no heat effect is produced when 5 per cent sodium pyrophosphate is added to CK with as much as 0.3 per cent water content, whereas the addition of calcium oxide to similar material may produce a rise in temperature of 4 C. The addition of calcium oxide to CK of higher water content may produce dangerous heat effects.^{4,5q}

The recommendations made in 1943^{17,18} as to the limits of impurities which should be allowed in CK for charging chemical warfare munitions (i.e., HCl, 0.005 per cent; water, 0.005 per cent; HCN, 0.02 per cent; and chlorine, none) proved to be unnecessarily stringent.

The present U. S. Army specifications for CK stored in 1-ton containers call for water, 0.5 per cent; hydrogen cyanide, 3 per cent; soluble residue, 0.02 per cent; chlorine, 0.005 per cent; acid (as HCl), 0.024 per cent; iron, 0.02 per cent; and CK assay, 96 per cent minimum.³⁷

Five per cent sodium pyrophosphate is added to the container prior to the addition of the CK. The specifications for 4.2-inch mortar shell charged CK also require the addition of 5 per cent sodium pyrophosphate to the shell prior to charging.³⁶

CK is noninflammable and there is thus no problem of ignition of the charging by bursting munitions as in the case of AC.

TABLE 2. Effect of water on stabilization of CK by sodium pyrophosphate.⁴

Conditions	Water content	Days for complete solidification	Stabilization in days
CK	0.03%	40, 40	
CK + 0.3 g steel (control)		7, 7	
CK + 0.3 g steel + 5% Na ₂ P ₂ O ₇		>209, >209	>202, >202
CK	0.23%	11, 12	
CK + 0.3 g steel (control)		7, 7	
CK + 0.3 g steel + 5% Na ₂ P ₂ O ₇		89, 89	82, 82
CK	0.53%	8, 12	
CK + 0.3 g steel (control)		3, 6	
CK + 0.3 g steel + 5% Na ₂ P ₂ O ₇		15, 15	9, 9

2.2.5 Detection and Analysis

Chemical methods for the detection of AC and CK are outlined in Chapter 34. Methods of analysis have been developed for the assay of plant run products with special reference to the impurities which may lower the stability of the agents on storage. The discussion of titrimeters in Chapter 37 includes the description of continuous recording instruments for AC and CK utilizing potentiometric titrations. NDRC Division 10 has developed recording instruments utilizing conductivity measurements and these instruments have been widely employed in the determination of concentrations of AC and CK in the field test programs.

AC is detectable by odor at about 0.03 g/l (i.e., 30 mg/l)¹³ on the average, although some men may lack almost completely the ability to detect the odor of cyanide. CK is readily detectable at about 0.02 mg/l both by its immediate lacrimatory effect and its irritant effect on the nasal passages.⁴⁴ At concentrations as low as 0.002 mg/l the eye irritation is noticeable by some observers in less than 3 minutes.

2.2.6 Canister Penetration

The dosages of AC and CK required for lethal penetration of captured German and Japanese canisters^{42,43} (1941-42 models) are about the same but the advantage lies with CK because of the higher dosages attained in the field.^c The higher liquid density of CK, which permits heavier bomb loadings, contributes to the higher dosages obtained per bomb. Lethal penetration of Japanese canisters by CK was obtained at dosages of 50,000 to 200,000 mg min/m³ at normal breathing rates. U. S. canisters filled with ASC charcoal give protection against several times these dosages. Field trials have demonstrated that dosages of CK in the range of 50,000 to 200,000 can be attained by feasible munition expenditures under favorable conditions of terrain and meteorology.⁴³

2.3 TOXICOLOGY

2.3.1 Toxicity

For the production of casualties among men prior to adjustment of the mask, the toxicity of the agent when breathed for 30 seconds, or perhaps 2 minutes in the case of sleeping troops, is the important characteristic. Against personnel without gas masks (e.g., in city populations) the toxicity of the agent over

longer periods is of importance. The toxicity over longer periods also comes into consideration in study of the physiological action of the concentrations penetrating canisters.

Ideally, the toxicity for man is the information desired. The data to be summarized in this chapter are based primarily on measurements of the action of the gases on various animal species and conclusions on the values of the agents are drawn therefrom. It should be noted, however, that in 1944 a thorough analysis of the problem of the indirect estimation of toxicities of AC, CK, and CG for man was made jointly by the Toxicological Research Laboratory of the Medical Division, Chemical Warfare Service, and the NDRC-University of Chicago Toxicity Laboratory.⁴⁰ For each of the three agents research programs were drawn up designed to provide indirectly the answer to the question of the concentrations required to cause death in man. Such an approach in the case of cyanide appeared particularly promising since certain basic data on the effect of cyanide on man are known from the open literature on the clinical use of sodium cyanide. The work on these programs is still in progress.

The detailed considerations of the probable effects of the three agents on man have led to a fuller understanding of the action of these nonpersistent gases and to more adequate interpretations of munitions trials. From the summary of the above-mentioned status report the sections on AC and CK are quoted to give the principles which enter into the attempt to make an indirect determination of the toxicities for man.

The purpose of this review was to define the laboratory experiments still required to reduce to a minimum the error in estimate of the concentrations of AC, CK, and CG required to cause death in man at different times of exposure.

A consideration of the tasks proposed for AC, CK, and CG discloses that concentrations of each agent lethal to man in 0.5, 1, 2, 10, 30, and 60 minutes are desired.

AC is known to be detoxified by animals and man. CK is detoxified by animals and so is presumably detoxified by man.

AC is detoxified by man at a rate of ca. 0.017 mg/kg/min when injected^d slowly.⁵⁶ This does not differ markedly from the rates at which it is detoxified by lower animals.⁵⁵

CK is detoxified by the rabbit, dog, and goat at rates of 0.03-0.06; 0.02-0.04; and 0.03-0.1 mg/kg/min respectively depending on the rate of injection.^{27,34a,b} Arguing by analogy with AC, man presumably will detoxify CK at a rate within these limits (0.02-0.1 mg/kg/min).

^d The experiments were made with NaCN, which upon injection into the tissues at about pH 7 yields HCN almost quantitatively.

^c See the Summary Technical Report of NDRC Division 10.

We have estimated that the LD_{50} of AC for man is ca. 1.1 mg/kg. This estimate rests on:

a. The LD_{50} of AC^e for six species of animals ^{6f,h} which indicates that the various species are not different in susceptibility, thus implying that man will fall in the same range.

b. Amounts of cyanide found in the tissues of humans committing suicide by taking cyanides.^{52,53}

c. The fact that the LD_{50} of AC bears an apparently constant ratio to the rate of detoxication of AC in various species.

The LD_{50} 's of CK for the rabbit, dog and goat are 3.15, 3.30, and 2.97 mg/kg respectively.²⁷ By analogy with AC man is probably equally susceptible.

Such evidence as is available indicates ^f that AC is equally toxic by inhalation or intravenous injection.^{6h} It is held in this review that this is also true for CK.

The minute volumes of different species of animals are stimulated differently by AC, 7-fold in the dog, 2-3-fold in the rabbit, and 1.5-fold in the guinea pig.^{6h} These volumes are sufficient to allow inhalation of an amount of AC approximately equal to the LD_{50} of AC for the several species.

Man's respiration is stimulated 7 to 10-fold by intravenously injected AC in single doses of ca. 0.055 mg/kg or more (estimated from reference 56).^g The duration of such stimulation is ca. 20 seconds.^h When infused slowly the percent stimulation is less (2-3-fold) but is longer maintained.

If CK is not more toxic by inhalation than by intravenous injection, then it too must stimulate the respiration of some species to allow the inhalation of a lethal dose. CK is known to stimulate the respiration of the dog (after causing apnea because of its irritancy).^{33a}

No satisfactory evidence is available to indicate that differences in the susceptibility of various species to AC and CK cannot be explained on the basis of difference in minute volumes in the presence of the gas, and the value of the intravenously injected LD_{50} .ⁱ

It is suggested that the toxicity of agents like AC and CK can be described as a first approximation by the formula

$$V\alpha C - Dt = K$$

in which

V = total volume of air breathed in l/kg

α = the fraction of inhaled gas absorbed

C = concentration in mg/l

D = rate of detoxication in mg/kg/min

^e Intravenous LD_{50} 's of AC in mg/kg (unanesthetized animals): dog 1.34, cat 0.81, monkey 1.30, rabbit 0.66, guinea pig 1.43, rat 0.81, and mouse 0.99. For anesthetized goats an LD_{50} of 0.66 has been obtained.²⁷ There are indications that the intravenous LD_{50} 's for anesthetized and unanesthetized animals are the same in some species ²⁷ but may not be identical in others.^{32c}

^f Later confirmed for dogs by the development of apparatus for direct measurement ^{39c} but found not to hold for rabbits,^{39d} where the LD_{50} by way of the lung proves to be about half of the intravenous LD_{50} .

^g This value for the respiratory stimulating dosage of injected NaCN has been checked in more recent studies on measurement of the velocity of blood flow in man.⁵⁷

^h Lesser stimulation but longer duration have been observed in legal executions with AC (reference 16 and later unpublished data).

ⁱ See exception in Note e.

t = time in minutes from first entrance of the substance into the body (roughly, the exposure time)

K = the lethal dose in mg/kg

The source of greatest doubt in estimating concentrations of AC and CK required to cause death in man from the above equation is the question of man's minute volume in the presence of the substance.

On the above basis, if a 70-kg man breathed 25 l of air during a 1-minute exposure to AC, the volume breathed per kilogram would be $25/70 = 0.36$ l. If 70 per cent of the inhaled AC is absorbed by the lungs, as experimentally determined for the dog,^{39c} the equation can be solved for the necessary lethal concentration of AC:

$$0.36 \times 0.7C - 0.017 \times 1 = 1.1$$

and

$$C = 4.4 \text{ mg/l.}$$

In this instance for the 1-minute exposure the $L(Ct)_{50}$ would be 4,400 mg min/m³. As has been pointed out, however, a meaningful estimate of man's minute volume in the presence of the gas is not readily made. It is upon earlier calculations of this type by British investigators that the internationally agreed estimates of 5,000 and 11,000 mg min/m³ for the $L(Ct)_{50}$'s of AC and CK are based.⁴⁴ As guides for use in munitions assessments the estimates have proved useful.

An alternative approach for use in determination of munitions requirements, developed from these data, places the emphasis on attainment of the minimum respiratory stimulating dosage rather than on the $L(Ct)_{50}$. This approach is based upon the fact that upon detection of AC a man will try to hold his breath long enough to adjust the mask. If the amount of gas which he inhales in the first breath is sufficient to stimulate the respiration, the probability of his receiving a lethal dose prior to adjustment of the mask is increased.

For a sedentary individual the volume inhaled per breath is about 0.6 l. If the first breath is to insure stimulation of respiration it should yield absorption of about $0.055 \times 70 = 3.9$ mg of AC per 70-kg man. If the absorption coefficient is 0.7, the concentration of gas to be aimed at in the gas cloud would be $3.9/(0.6 \times 0.7) = 9.3$ mg/l.

It can be seen from this analysis that the prediction of the toxicity of AC for man, if feasible, is a problem requiring knowledge of the mechanism of the action of AC, its inherent toxicity per kilogram of body weight in different species, its influence on the rate and volume of respiration, the per cent of

TABLE 3. $L(Ct)_{50}$'s of AC for different species.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³) or suggested value where number of animals is small	A = anal. conc. N = nom. conc.	Number of animals used	Reference
Mouse	$\frac{1}{2}$	400	A	25	11
	$\frac{1}{2}$	500	A	44	2
	1	900	A	44	2
	1	600	A	50	11
	2	1,300	A	95	6a
	2	1,280	A	260	15
	2	1,160	N	160	6f
	2	1,280	N	160	6f
	2	1,320	N	80	6f
	3	1,100	A	30	11
	10	2,300	A	300	12
	30	5,250	N	...	6g
	30	5,600	A	180	15
Rat	$\frac{1}{2}$	800	A	24	2
	1	1,550	N	76	6h
	2	2,200	A	100	23
	3	1,800	A	18	11
Guinea pig	$\frac{1}{2}$	2,500	A	28	2
	1	2,100	N	60	6h
Rabbit	1	850	N	32	6e
	10	3,200	A	21	11
Cat	1	850	N	30	6e
Monkey	1	1,700	N	10	6e
Dog	$\frac{1}{2}$	800	A	30	11
	1	700	N	24	6e
	1	700	A	26	11
	3	1,000	A	26	11
Goat	$\frac{1}{2}$	1,300	A	20	31
	2	2,200	A	18	24

the gas which is absorbed by the lungs, and its rate of detoxication. The outlay of research required to gain an approach to the actual toxicity for the human species is an effort which has been attempted only for a few agents of primary importance. In general the screening of possible new war gases has rested on the routine determination of the $L(Ct)_{50}$'s for various animal species. Most of the toxicological conclusions on new agents have had to rest on comparison of animal $L(Ct)_{50}$'s, combined with qualitative evaluation of the effects of the different agents on man.

The data on the $L(Ct)_{50}$ values of AC and CK for different species are summarized in Tables 3 and 4. Where the number of animals used in the determination is small, the $L(Ct)_{50}$ is only an approximation. In these tables the values which have a fairly substantial experimental basis have been included. The tables include World War I and World War II data. A fuller tabulation is given elsewhere.⁴⁰

A comparison of the $L(Ct)_{50}$'s of AC and CK for different species is given in Table 5 for exposure times of 1 to 2 minutes. Since the density of the gas

governs the weight of filling per bomb of given volume, the last column in the table represents the efficiency in terms of munition chargings. From Table 5 it is seen that for most of the species AC is several times as effective as CK in short exposure periods. For long exposures the ratio of the toxicities approaches a value close to the inverse ratio of the cyanide radical contents of the two gases.

The course of respiration of goats during exposures to AC and CK has been studied in detail at Dugway Proving Ground. During exposure to AC for 2 minutes, respiration was normal for the first few seconds.^{21a} At concentrations above 2.5 mg/l the time of onset of stimulation of respiration was 10 to 20 seconds after the start of the exposure. The duration of stimulation was 30 to 100 seconds and was followed by a depression of respiration setting in at 40 to 150 seconds. In similar experiments with CK at concentrations above 2 mg/l respiration is irregular and depressed during the first 30 to 80 seconds.^{21c} The breath is not actually held, however, as in the case of goats exposed to phosgene (CG).^{21b,31} Fol-

TABLE 4. $L(Ct)_{50}$'s of CK for different species.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³) or suggested value where number of animals is small	A = anal. conc. N = nom. conc.	Number of animals used	Reference
Mouse	$\frac{1}{2}$	3,000	A	50	44
	1	4,550	N	160	6b
	...	3,640	A	...	...
	1	4,200	A	29	44
	2	5,600	N	120	6f
	2	5,300	N	100	3
	2	6,200	A	180	14
	2	3,600	A	70	44
	3	4,200	A	40	44
	10	7,900	N	200	3
	10	7,500	A	160	14
	30	13,500	N	180	3
	30	13,800	A	140	14
Rat	1	13,000	N	35	3
	2	9,400	A	30	44
	3	5,400	A	20	44
	$7\frac{1}{2}$	6,300	A	18	10
	30	9,000	A	16	10
Guinea pig	1	15,000	N	35	3
	2	7,000	A	30	44
	$2\frac{1}{2}$	5,500	A	16	10
	$7\frac{1}{2}$	9,000	A	13	10
	30	17,000	A	13	10
Rabbit	1	13,000	N	10	3
	2	8,000	A	24	44
	$7\frac{1}{2}$	6,000	A	23	10
	30	17,000	A	20	10
Cat	1	6,000	N	10	3
Monkey	1	4,400	N	20	3
Dog	1	3,800	N	26	3
	3	4,200	N	18	3
	$7\frac{1}{2}$	4,500	A	26	10
	10	5,000	N	26	3
	30	6,000	N	14	3
Goat	2	3,600	A	30	25

lowing the period of depression, there is a stimulated phase of 40 to 90 seconds' duration and this result is in accord with the conversion of CK to AC in the body, as described in Section 2.3.3. There follows a variable period during which the respiration again falls below the basal rate. With both AC and CK

this later period of depression is followed by complete respiratory paralysis, usually within a few minutes, in those animals receiving lethal dosages and by gradual return to normal in those receiving sublethal quantities of the agents.

Since CK yields AC in the body, a 50/50 mixture

TABLE 5. Comparison of $L(Ct)_{50}$'s of AC and CK for short exposure times.

Species	Exposure time (min)	$\frac{L(Ct)_{50} \text{ of CK}}{L(Ct)_{50} \text{ of AC}}$	$\frac{L(Ct)_{50} \text{ of CK}}{L(Ct)_{50} \text{ of AC}} \times \frac{\text{Density of AC}}{\text{Density of CK}}$
Goat	2	1.6	0.9
Monkey	1	2.6	1.5
Mouse	1	4.0	2.3
Dog	1	5.4	3.1
Cat	1	7.0	4.1
Guinea pig	1	7.1	4.1
Rat	1	8.4	4.9
Rabbit	1	15.3	8.9

of AC and CK should show a toxicity which is a function of the combined effect of the cyanide contributions from both agents. In a series of determinations of the toxicities for mice of AC diluted with increasing percentages of CK, the 2-minute $L(Ct)_{50}$ was 1,250 for pure AC, 1,880 for 50/50 AC-CK, and 5,400 for pure CK.^{6f} Calculated on the basis of cyanide radical content the values are, respectively, 1,200, 1,300, and 2,300. Part of the lower effectiveness of CK on a cyanide content basis is to be accounted for by the tendency of the irritant vapors of CK to depress respiration during the first part of the exposure period. That there is reinforcement between the two agents is evidenced by the fact that the 50/50 mixture is essentially as toxic as pure AC on a cyanide radical basis.

AC vapor is absorbed slowly through the skin. Experiments on body exposure during which the animals breathed uncontaminated air indicated that mice were killed at 10-minute Ct 's of about 200,000 mg min/m³, cats at 500,000, and dogs at 1,000,000.² The order of the sensitivity in these tests is to be expected on the basis of an increase in the required Ct with decrease in the ratio of body surface to body weight. The results serve to indicate that for man the required Ct is of such a magnitude that it does have significance in the consideration of AC as chemical warfare agent.

The irritancy of CK vapor to the human skin has been noted by personnel engaged in field tests with this agent. In a series of chamber experiments under controlled conditions groups of men wearing masks were exposed to several concentrations of CK.⁴⁹ Under hot and humid conditions the irritation, primarily in the genital region, became severe at 2.0 mg/l. Under temperate conditions similar effects were obtained at about 3.6 mg/l. The irritation, although severe, is transient and does not persist after removal from the CK-containing atmosphere.

The LD_{50} 's of AC and CK by intravenous injection have already been cited. When administered orally or by stomach tube, the rate of absorption of the agents from the digestive tract affects the LD_{50} .⁵² In orally administered doses of NaCN sufficient to kill dogs within less than 10 minutes, as much as three-fourths of the administered cyanide is found in the gastrointestinal tract after death. In dosages yielding later deaths the per cent absorption is higher. Calculated on the basis of *absorbed* HCN, the LD_{50} orally administered is not significantly different from the intravenous value. The LD_{50} 's for ocular and for

nasal administration of AC in cats are also found to be close to the intravenous value.^{6g} The LD_{50} of CK administered in aqueous solution by stomach tube has been found to be approximately 6 mg/kg with deaths occurring at about 30 minutes.²⁶

2.3.2

Pathology

In animals dying from acute cyanide poisoning pathological examination shows evidence of marked tissue anoxia. Hemorrhages are most apparent in the thymus glands.² Pathological studies from World War I and from World War II indicate that residual lesions from AC are significant only in the case of animals receiving an exposure in a narrow range just below the minimal lethal dose, resulting in irreversible injury to the more susceptible nervous tissue but failing to cause acute respiratory paralysis and death of the animal.⁴⁹ Nearly all animals recovering from sublethal doses do not experience significant tissue anoxia and are free from any demonstrable after-effects. In the animals showing residual neurological damage the principal pathological changes are noted in the cerebrum and the cerebellum.^{2,29}

Residual paralysis following CK exposures is similar to that obtained with AC and is observed more frequently in dogs than in other species.^{6c} In addition CK has a local irritant effect on lung tissue. In occasional instances the lung irritation can lead to pulmonary edema,^{28,44} which may be important from the therapeutic standpoint. From the offensive standpoint the pathological results show that the immediate paralyzant effect of CK greatly overshadows its other effects.⁴⁴

Mice appear to be more resistant than dogs to lung irritation from CK. Mice surviving 35 exposures to nearly lethal dosages of CK showed no gross pathological lesions in the lungs.^{6d}

2.3.3

Physiological Mechanism

Hydrocyanic acid exerts its lethal action through inhibition of cellular respiration producing an asphyxia leading to death. The agent causes a temporary increase in respiratory volume as a result of action on the carotid body; if the carotid body is removed, only respiratory depression is obtained. The therapeutic value of methemoglobin is a result of its strong affinity for cyanide, in competition with cytochrome oxidase, yielding a nonionized and nontoxic combination.

In extension of the basic information on the mech-

anism of action of AC, the competition for cyanide between methemoglobin and cytochrome oxidase has been demonstrated *in vivo*.^{32b} The therapeutic value of methemoglobinemia induced by nitrates and by *p*-aminopropiophenone has been thoroughly studied by Medical Division, Chemical Warfare Service, and by the Office of Scientific Research and Development [OSRD] Committee on Medical Research. Cyanide is detoxified by gradual excretion as thiocyanate and the therapeutic value of thiosulfate has been further investigated. From the practical standpoint the deaths from exposure to gaseous AC are rapid and under battlefield conditions there would be few opportunities to apply therapeutic procedures of this type in time to be of value. The production of methemoglobinemia among large groups of troops as a protective measure prior to possible exposure has been considered but is not a practical procedure in the field and would reduce the efficiency of all troops thus treated.³⁰

It has been demonstrated that cyanogen chloride is converted to hydrocyanic acid in the body and exerts its lethal effect as AC. Some of its other toxicological properties such as irritancy to the nasal passages and local action on lung tissue are characteristics of CK itself. The similarities in the actions of CK and AC were pointed out by British investigators⁴⁵ in the reporting of the conversion of CK to AC in whole blood in a matter of seconds. Blood serum alone does not accomplish the conversion. The reaction is not a simple one and proceeds in two stages.⁴⁶ CK reacts with hemoglobin to give a compound which in the presence of reduced glutathione yields AC. In connection with the mechanism of the first stage it has been demonstrated that CK is capable of reacting with both amino groups and sulfhydryl groups of amino acids and proteins.⁹

Following the British work the observations were

extended to additional animal species, to blood *in vitro* and *in vivo*, and to human blood.^{39a} The conversion is not quantitative. The maximum conversion by human red cells was 86 per cent and the average was considerably lower than this value. About 75 per cent conversion has been found *in vivo* in the rabbit.^{32c} No free CK is present, however. The fate of the fraction which does not appear as HCN has not been determined.

Although a slow combination of CK with methemoglobin can be demonstrated when the agent is present in great excess, the slowness of this combination compared with the rate of the conversion reaction to AC makes it unlikely that the combination of CK with iron compounds such as methemoglobin has any significance *in vivo*.^{39b} Induced methemoglobinemia is effective therapeutically against CK^{32a,33b} in agreement with the data on conversion to AC.

2.4 EVALUATION AS WAR GASES

For the task of production of immediate deaths by the attainment of effective dosages in less time than is required for masking, AC is considered from the toxicological standpoint to be the best agent available.^{41,43} Its competitors are the almost equally quick-acting but very much less volatile fluorophosphates and analogs, one of which was put into production by the Germans (Chapter 9). The tendency of AC to flash in some standard munitions is a disadvantage which has not been overcome.

For the task of penetration of World War II models of German and Japanese canisters under favorable conditions of terrain and meteorology, CK was the most suitable agent. It can be satisfactorily stabilized and is nonflammable. It is also second choice to AC in the task cited in the preceding paragraph.

Chapter 3

PHOSGENE^a

By *Stanford Moore* and *Marshall Gates*

3.1 INTRODUCTION

PHOSGENE (CG) was the principal nonpersistent gas of World War I and was the standard nonpersistent gas filling for United States bombs and mortar shell at the outbreak of World War II. Throughout World War II the stocks of CG were maintained at a much higher level than those of AC and CK, which were later standardized as quick-acting nonpersistent agents. When delayed physiological effects are acceptable, CG has been considered the most economical standard nonpersistent agent for the production of casualties by attainment of effective dosages in less time than is required to mask or for production of casualties among unprotected personnel.³⁰

This chapter summarizes primarily the recent advances in the information on the toxicological action of CG. Data on the chemistry and toxicology of diphosgene and carbonyl chlorofluoride are also included. These two agents parallel CG in toxic action but have not been considered to possess any major advantages over CG as chemical warfare agents.

3.2 PREPARATION AND PROPERTIES

3.2.1 Preparation and Stability

CG has been prepared industrially for many years by the direct combination of carbon monoxide and chlorine under the catalytic influence of activated carbon. The product is stable and long experience has shown that CG can be stored indefinitely in iron and steel containers in the absence of moisture.

3.2.2 Physical Properties

The physical properties of CG which have the most direct bearing on the use of this gas as a warfare agent are listed below (see Chapter 2 for properties of AC and CK).

Density (liquid) g/ml at 20 C	1.38
Boiling point, C	8.3
Freezing point, C	-104
Heat of vaporization, cal/g	60
Vapor density, relative to air	3.5

^a Based on information available to Division 9 of the National Defense Research Committee [NDRC] as of December 1, 1945.

CG possesses several advantages over AC and CK in its physical properties. It has a higher liquid density and a lower boiling point than AC or CK. The freezing point of CG is so low that the agent will remain liquid at any temperature to be encountered in operations. From the standpoint of gas cloud behavior, the effect of the lower heat of vaporization of CG is offset in part by its greater vapor density. Field trials show little difference between the travel of CG clouds and that of CK clouds.³¹

3.2.3 Detection and Analysis

Chemical methods for the detection and analysis of CG are outlined in Chapter 34 of this volume.^b The median detectable concentration by odor is given as 0.006 mg/l.²¹

3.2.4 Canister Penetration

The dosage of CG required for lethal penetration of the more recent models of German, Japanese, or Allied canisters is 500,000 to 1,000,000 mg min/m³ under conditions favorable for penetration and as much as twice these figures for humidified canisters at low breathing rates.³¹ In general, dosages in this range are beyond those which can be obtained over a significant portion of a target area by feasible expenditures. Thus both Allied and enemy canisters provide good protection against this agent.

3.3 TOXICOLOGY

3.3.1 Toxicity

CG exerts its lethal action by injury of the lung tissue in contrast to AC and CK, which are systemic poisons. The rate of detoxification is so low that it comes into consideration only in the study of defensive measures against long exposures to trace concentrations in manufacturing plants. An approach to the problem of the toxicity of CG for man differs from that applied to the systemic poisons. The differences in the susceptibility of the various species to CG have been attributed²⁹ to the following factors which govern the extent of injury to the lung tissue:

^b See also the Summary Technical Report of Division 10.

TABLE 1. $L(Ct)_{50}$'s of CG for different species.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³) or suggested value where number of animals is small	A = anal. conc. N = nom. conc.	Number of animals used	Reference
Mouse	1	3,450	N	240	9
	1	6,300*	N	300	9
	2	4,700	A	180	22
	3	1,950	N	200	9
	10	1,800	N	220	9
	10	3,800	A	100	20
	20	2,000	N	280	9
	30	3,400	A	160	22
Rat	1	6,500	N	24	9
	30	1,400	N	32	17
Guinea pig	1	2,800	N	28	9
	30	1,300-2,200	N	30	17
Rabbit	30	1,000	N	30	17
Monkey	1	600-1,000	N	13	9
	5	625	A	21	39
	10	750	A	25	39
	30	1,000	N	14	17
	0.5	8,100	A	28	18
Dog	1	8,400	A	28	18
	1	7,000	N	9	9
	3	4,500	A	29	18
	5	4,500	N	16	9
	5	4,250	A	29	18
	20	4,200	N	19	9
	2	4,600	A	14	28
	2	6,500	A	72	27
Horse	ca. 10	ca. 10,000	A	23	34

* Determination on Jackson strain of mice. Other OSRD data are on Carworth male mice.

1. Differences in the amount breathed during exposure.

2. Differences in the depth of inhalation and the size and shape of the upper respiratory tract, leading to a relatively greater absorption of agent in the upper respiratory tracts of the smaller species.

3. Minor differences in tissue susceptibility.

4. Differences in resistance to death from the prolonged anoxia resulting from pulmonary edema, such differences being known to be present even within a given species with variations in nutritional state and general health of the animals.

The factors involved in the toxic action of CG are such that the differences in the sensitivities of different species to this agent are more difficult to interpret than in the study of AC and CK. $L(Ct)_{50}$ measurements for different species are summarized in Table 1. The relative $L(Ct)_{50}$'s of CG, AC, and CK are given in Table 2.

The data show the effect of a depression of respiration during short exposures to CG.³⁵ The 1-minute $L(Ct)_{50}$'s are in most cases several times the 10- or 30-minute figures. In the case of the goat it was not

possible to determine the $L(Ct)_{50}$ for a 30-second exposure time since the animals frequently held their breath during the complete period in the presence of high concentrations.²⁷ The depression of respiration in this species was actually characterized by breath holding and was more marked than the action of CK, which under similar conditions induced depressed respiration but not complete cessation. This reflex respiratory inhibition evoked by CG has also been studied in detail in the dog.⁹ At concentrations higher than 7 mg/l the first inhalation of the phosgenized atmosphere caused total cessation of respiration, with the lungs in the deflated phase. The duration of this apnea averaged 26 seconds. For exposure periods of 1 minute the average reduction of lung output was 62 per cent. If the vagus nerves were cut prior to exposure, no reflex inhibition of breathing was obtained. In the case of the monkey the $L(Ct)_{50}$ data give no indication of breath holding during a 1-minute exposure (Table 1). The monkey is by far the most sensitive species tested with CG and the lethal concentration (0.6 to 1.0 mg/l) in this case appears to be below the level capable of inducing the reflex

TABLE 2. Comparison of $L(Ct)_{50}$'s of CG, AC, and CK.

Species	Exposure time (min)	$\frac{L(Ct)_{50} \text{ AC}}{L(Ct)_{50} \text{ CG}}$	$\frac{L(Ct)_{50} \text{ AC}}{L(Ct)_{50} \text{ CG}} \times \frac{\text{Density CG}^*}{\text{Density AC}}$	$\frac{L(Ct)_{50} \text{ CK}}{L(Ct)_{50} \text{ CG}}$	$\frac{L(Ct)_{50} \text{ CK}}{L(Ct)_{50} \text{ CG}} \times \frac{\text{Density CG}}{\text{Density CK}}$
Mouse	1	0.26	0.52	1.3	1.5
	30	1.6	3.2	4.0	4.6
Rat	1	0.24	0.48	2.0	2.3
	30	...	...	6.4	7.4
Guinea pig	1	0.75	1.50	5.4	6.3
Monkey	1	1.7	3.4	4.4	5.1
Dog	1	0.10	0.20	0.45	0.52
	3	0.22	0.44	0.93	1.1
	30	...	...	1.4	1.6
Goat	2	0.34	0.68	0.55	0.64

* Calculated for comparison on a per bomb basis.

action. In the dog little or no apnea was produced by exposures to 0.65 and 1.2 mg/l of CG.⁹

It was shown following World War I¹⁹ that in tracheotomized dogs inhaled CG was 60 per cent absorbed during 7½-, 10-, 15-, and 30-minute exposures. Based on the CG absorbed, the LD_{100} was about 0.74 mg/kg. This value, when compared with the LD_{50} of 0.95 mg/kg for absorbed AC in the dog (see Chapter 2), would indicate that for this species CG is intrinsically more toxic than AC. On the other hand, the approximate 30-minute $L(Ct)_{50}$'s of AC and CK for the dog, which involve close to the same volume breathed, indicate that AC is slightly more effective on an LD_{50} basis than is CG for this species. But it is striking to note in Table 2 that on the basis of the dosages to which dogs are *exposed* (not the absorbed dosage given above) AC is ten times as toxic as CG in short exposure periods (1 minute). This result is a function of the manyfold increase in minute volume induced by AC coupled with the depression of respiration produced by CG. These figures illustrate the importance of respiratory volume in any attempt to estimate the toxicity of CG for man.

With a possible exception in the case of AC in the dog, for longer exposure periods such as 30 minutes CG is more toxic than AC or CK. The $L(Ct)_{50}$'s of the cyanide agents increase as a result of the detoxification rate and the differences in respiratory volume decrease in significance as the time is lengthened.

The variations in sensitivity to CG within a given species are evidenced by the data on mice in Table 1. Two strains of mice were exposed under the same conditions in the same laboratory and gave widely differing $L(Ct)_{50}$'s. It is not certain whether the sensitivity is purely a function of strain. The Jackson mice were tough, scrawny, and extremely active and the Carworth mice were relatively fat and glossy,

averaging 2 to 4 g heavier than the Jackson strain. From several sources there is evidence that within a given strain animals that have been deprived of food or water for a period before exposure are more resistant to CG.^{12a,b,14b,33} To cite one example, Carworth mice placed on a restricted diet leading to a weight loss of about 15 per cent were gassed for 10 minutes along with a group of control animals allowed to feed *ad libitum*. Mortality in the restricted group was only 4/30 compared with 13/29 in the controls.⁹ An abrupt change in environmental temperature was found to be another factor which affected the resistance of mice to CG.⁹ Abnormal post exposure temperatures led to increased sensitivity. Effects of these types, whether they be due to dehydration, fasting, strain, or temperature, probably account for the apparent discrepancies among mouse $L(Ct)_{50}$ determinations from different laboratories (Table 1).

The comparison of the $L(Ct)_{50}$'s of CG, AC, and CK in Table 2 shows that for short exposures CG is only one-fifth as effective as AC against the dog and is more than three times as effective as AC against the monkey. There is no evidence which permits establishment of the relative $L(Ct)_{50}$ for man. For the calculation of munition expenditures the Allies have employed the value of 3,200 mg min/m³ for CG in comparison with 5,000 for AC and 11,000 for CK. Taking into consideration the relative liquid densities of the three agents, the toxicity estimates which have been used on a per bomb basis are in the order of 1/3/4. It will be noted that the ratios of these values are similar to the ratios given for the monkey in Table 2.

An estimate of the relative values of the toxicities of CG and the cyanide agents is only a part of the picture. The relative suitabilities of the three agents

in the field would also be a function of additional important considerations. CG kills only after a delay of a number of hours, whereas AC and CK were standardized as "quick-acting" nonpersistent agents. CG also produces serious casualties in sublethal dosages and individuals may require hospitalization for several weeks prior to recovery. AC and CK in general produce no extended disability if the quantity inhaled is less than the lethal dosage. Injurious sublethal dosages of CG may be received prior to masking or after masking in the presence of high concentrations of CG if hurried adjustment of the facepiece leads to the presence of small leaks.

For the production of casualties by attainment of effective dosages in less time than is required to mask, the toxicological data point to the working hypothesis that CG is the preferable agent if delayed effects are acceptable.³⁰ This estimate takes into consideration both deaths and disablement of troops from sublethal dosages. If the tactical situation requires immediate deaths, the choice lies only with AC and CK. For harassment CG is considered the most satisfactory nonpersistent agent in view of its disabling action in sublethal amounts.

Studies have been made of the effects of breathing low concentrations of CG for long periods to determine the health hazards from trace concentrations which might be encountered in manufacturing operations. British investigators exposed animals to a concentration of 0.0044 mg/l (1/1,000,000) for 5 hours on 5 successive days. Microscopic findings showed that all the animals were affected by CG to a degree likely to give rise in man to serious clinical symptoms.³² The experiments were repeated at a CG concentration of 0.0009 (1/5,000,000). Evidence of slight pulmonary edema and bronchitis was observed even at this low concentration.³³ It was concluded that at this concentration the limiting level of safety has approximately been reached. It will be noted that 0.0009 mg/l is below the median detectable concentration by odor measured with the osmoscope.²¹ This does not necessarily mean that dangerous concentrations of CG may be undetectable by odor. The osmoscope values are useful for laboratory comparison of the detectability of different gases. It is known, however, that in free air, where an *average* low concentration is actually present in instantaneous peaks and valleys of concentration, a person is aware of the presence of mustard gas, for example, at average concentrations much below the osmoscope value.

3.3.2

Pathology

The basic data on the pathology of phosgene poisoning were reported following World War I.⁴⁷ The subject was reviewed in 1943²³ with reference to the observations made since that time, including studies by the Medical Division, Chemical Warfare Service, and the Committee on Medical Research [CMR] of the Office of Scientific Research and Development [OSRD]. The most recent CMR data on the pathology are summarized elsewhere.^{12c,4c} In man and experimental animals the factor initiating the pathological changes in the lungs is bronchiolar injury. Gassed patients die most frequently in the second half of the first day in pulmonary edema, with or without peripheral circulatory failure. Residual effects of phosgene poisoning in human subjects have been summarized by the Medical Division.²⁶

3.3.3 Physiological Mechanism and Therapy

CG injures lung tissues by virtue of combination with cell constituents. With the simpler amino acids, for example, the reaction product is an amino acid ureide, $O = C(NHCHRCOOH)_2$. Investigations by the Committee on Medical Research have shown that CG is capable of reacting under physiological conditions with $-NH_2$, $-SH$, and $-OH$ groups of amino acids, peptides, and proteins.^{12c,13,14c} The reactivity of CG towards proteins produces in enzymes and hormones irreversible inhibitions of their enzymatic and hormonal activities.^{13,14c} Experiments with CG containing radioactive carbon¹⁶ have established the fact that a significant percentage of the inhaled agent is bound locally in the lung tissue, paralleling the results on the tissue fixation of radioactive mustard (see Chapters 22 and 23).

The early theory that CG might exert its toxic action by virtue of the hydrochloric acid liberated intracellularly on hydrolysis has been shown to be untenable.^{14a} Part of the evidence is the parallelism between the toxic action of ketene ($CH_2 = C = O$) and CG. Ketene kills animals with the same clinical picture of lung edema and with the same histological injury to the lungs as is produced by CG. The $L(Ct)_{50}$'s of ketene for different species are of the same order of magnitude as those of CG.¹⁰ Ketene, however, produces no mineral acid on hydrolysis. Also hexamethylenetetramine serves as a prophylactic agent against ketene as well as against CG, the prophylactic action resulting from competition for the toxic agents between the hexamethylenetetramine

and the amino or other reactive groups of the cell constituents.^{14a}

The extensive studies on the treatment of CG casualties have been reviewed^{15,23,24} and summarized.²⁵ Agents such as hexamethylenetetramine are effective if administered prior to exposure but have no practical application to the soldier in the field. The knowledge on the general treatment of pulmonary edema has been extended through studies on the application of oxygen therapy under positive pressure in the case of CG casualties. In general no procedures have been found which have therapeutic value specifically effective against phosgene poisoning as distinguished from pulmonary edema of different etiology.

3.4 EVALUATION OF CG AS A WAR GAS

When delayed physiological effects are acceptable, CG has been considered the most economical standard nonpersistent agent for the production of casualties by attainment of effective dosages in less time than is required to mask or for production of casualties among unprotected personnel.³⁰

3.5 DIPHOSGENE

Trichloromethyl chloroformate, diphosgene (DP), was used as a combat gas by the Germans in World War I. The fact that DP could be filled into ordinary HE shell by the simple expedient of cementing the joints was of importance to the Germans.⁴⁵ With the development of special shell and bombs for plant filling with CG the interest in DP became less. For the task of production of lethal dosages in 30 seconds or 1 minute the more volatile CG was preferable. Interest in DP was temporarily renewed in World War II when it was shown that DP could be rapidly converted catalytically to CG in shell and a study was made of whether plant filling with DP followed by conversion to CG in the munition would be a useful procedure. The advantages of this method did not appear to offset the increased production costs for DP and the modifications of standard munitions required in some instances. The more recent data on the chemistry and toxicology of DP are included in the following brief summary of work on this subject.

DP boils at 127.5–128 C at atmospheric pressure and freezes at –57 C.⁴⁶ Its specific gravity is 1.645 at 20 C,² and its vapor density relative to that of air is 6.9. DP reacts with aniline in aqueous solutions or in benzene to give quantitative yields of carbanilide.

This reaction may be used for detection and analysis.⁴⁶

DP was first prepared by Hentschel in 1887⁴² by the chlorination of methyl formate in direct sunlight. Methyl chloroformate was employed as a starting material by the Germans in World War I. Thorough studies by the French^{40,41,44} indicated that the photochlorination of methyl formate proceeds with increasing difficulty as the number of hydrogen atoms replaced increases, and that the last stage is slow unless adequate illumination rich in the shorter wavelengths is used. It is possible to conduct the later stages of the chlorination at higher temperatures, although the French workers recommended a temperature not exceeding 90 C, since decomposition of the product to CG becomes appreciable at higher temperatures.

The direct chlorination of liquid methyl formate leads to considerable charring and may become violent. To avoid undue loss of the volatile methyl formate, very efficient cooling of the by-product gas must be maintained. To minimize these difficulties, chlorination can be carried out in dilute solution, diphosgene itself being a convenient solvent.² Using a laboratory batch process employing this solvent and internal illumination from low-pressure discharge tubes, methyl formate can be converted into excellent quality DP in yields of 92 per cent on the ester and 90 per cent on the chlorine.² In this process it is necessary to carry out the final stages of chlorination at 80 C because of the low velocity of the last chlorination step. This temperature appears to be about the maximum which can be used without prohibitive losses of DP through conversion to CG.²

A continuous process based on the above method has been operated on a pilot plant scale.⁷ A total of about 1,000 lb of DP was produced in 86 per cent yield on the ester, 55 per cent on chlorine, by this pilot plant, which consisted essentially of two 5-l reactors in series, each reactor being illuminated by a well containing a 200-w projection lamp. Liquid methyl formate and gaseous chlorine were introduced into DP in the first reactor, which was operated at 50–55 C and received 80 per cent of the chlorine input. The overflow from this reactor was led to the second reactor, which was maintained at 75–80 C and which received 20 per cent of the chlorine input. The product overflowed from the second reactor and was cooled and scrubbed with dry air to remove HCl and Cl₂. All stages of the reaction are exothermic, and the capacity of the unit appeared to be limited

by the rate at which heat could be dissipated from the first reactor, in which most of the chlorination occurred. Rough estimates indicate that 121 kcal must be removed per mole of methyl formate used. A program to determine design factors for a large-scale plant was not completed.⁷

A continuous two-stage process has been developed by the Canadians^{33a} for the photochlorination of methyl formate to DP. It is similar to the above process in that DP is used as a diluent but different from it in that chlorine concentrations approaching saturation are used, better provision for the dissipation of heat during the initial stages is provided, and the charge is allowed to remain considerably longer in the second stage. In agreement with earlier work, the reaction was found to have a high temperature coefficient and to be promoted by light of wavelength shorter than 5300 Å. The violet mercury line of commercial "blue neon" discharge tubes proved to be highly effective as a light source. The process gives DP in 85 per cent yield based on methyl formate, and has been operated on a pilot plant scale to yield 2 lb per hour of good quality DP in a yield of 87.5 per cent on methyl formate and 68.4 per cent on chlorine.³⁶

Commercial chlorine contains some substance, possibly oxygen, which markedly inhibits the chlorination of methyl formate.^{2,36,38} Venting of the chlorine cylinders used removes this impurity, and satisfactory chlorination results thereafter, although the loss of chlorine may be considerable.³⁶

Decomposition of DP to CG can be effected by heat. The reaction is accelerated by activated carbon and some metallic halides.^{40,41,43} Work carried out in this country in 1941 indicated that in addition to the known thermal and contact-catalyzed decomposition of DP to CG, this decomposition was remarkably susceptible to catalysis by organic bases.^{1,4} A number of liquid amines, e.g., pyridine, produce a violent and almost instantaneous conversion. The suggestion was made that bombs could be charged with DP and stored as such, the catalytic conversion being made to proceed rapidly by the use of pyridine as catalyst after the release of the bomb. A preliminary model of a device by which this might be accomplished was designed and constructed.¹ However, the production of a bomb incorporating such a device presents several difficulties, and the advantages were not considered sufficient to merit further research and development. Solid amines, such as Michler's ketone, produce a slower decomposition,

the rate of which can be in part controlled by controlling the proportion of catalyst, its rate of dissolution, and the temperature. It was suggested that gas shell could be charged with DP, which is simpler than filling with low-boiling CG, a pellet of catalyst containing Michler's ketone added, and the shell closed, after which conversion to CG would take place. Laboratory experiments indicated that this conversion would be substantially quantitative and complete within an hour.

In attempting to work out the details incident to the use of solid catalysts for the conversion of DP to CG in shell, it was soon discovered that temperature effects play a critical role in the course of the reaction. If the catalyst pellet disintegrates rapidly, the strongly exothermic dissociation of DP quickly warms the liquid to a certain critical temperature, after which the decomposition proceeds very rapidly and to completion. If, however, the pellet disintegrates slowly, or the temperature and heat capacity of the bomb are such that this temperature is not reached, the conversion is never complete and is usually not over 20 per cent. Small-scale experiments in glass vessels are thus valueless for the prediction of the behavior of DP in actual munitions on the addition of dissociation catalysts. Experiments made with a metal bomb whose weight and void closely approximated those of a 105-mm shell indicate that a catalyst pellet composed of 2 parts Michler's ketone, 5 parts *p*-dichlorobenzene, 1 part paraffin wax, 4 parts red lead when used in an amount equal to 1 to 1.5 parts per thousand of DP will produce satisfactory conversion. Under these conditions, maximum temperatures of 42–43°C, starting with the bomb and contents at 25°C, are reached, and momentary pressure surges of 250–275 psi, dropping to 30–35 psi after 24 hours, are observed.⁴ The function of the *p*-dichlorobenzene and the paraffin in the catalyst is to prevent too rapid disintegration of the pellet; that of the red lead is to increase the density so that the pellet will not float on the DP.

The phenomena which occur after the addition of such a pellet to a sample of pure DP are striking. Michler's ketone forms with CG a deep blue addition compound. On addition of the pellet a faint layer of blue appears on the surface of the DP, and the red pellet turns almost black. After several minutes the pellet begins to disintegrate rapidly, and the liquid rapidly becomes colored an intense blue. During this period there is a slight evolution of gas, which suddenly becomes violent and continues until the

liquid has almost completely boiled away. The start of the violent ebullition, which can be determined on duplicate samples within 20 seconds, begins when the sample reaches 34 C and under controlled conditions can be used as a criterion of purity for DP.^{4,7,36,38a} The presence of hydrogen chloride in the DP increases its "ebullition time" considerably.

When liquid DP is decomposed in this way in an open vessel, insufficient heat is produced to overcome the latent heat of vaporization of the CG produced, and, in spite of an initial rise in temperature, the temperature of the liquid eventually falls below the boiling point of CG. If pyridine in amounts equal to 10–11 per cent of the weight of DP is used to catalyze the decomposition, calorimetric experiments indicate that the heat produced is approximately equivalent to that required to overcome the latent heat of the phosgene produced.^{38b} Several rough experiments indicated that it may be possible to produce instantaneous clouds without cooling the immediate atmosphere by the simultaneous mixing and bursting of DP plus 10–11 per cent pyridine.^{38b} In view of the important contribution of the local inversion produced by the cooling effect, which aids in the attainment of high dosages with nonpersistent agents, it is doubtful whether the production of a gas cloud with no fall in temperature would have advantages at air temperatures above the boiling point of CG.

In aqueous solution DP yields 2 moles of CG.⁴⁶ Much of the work on the mechanism of action of CG (see Section 3.3.3) has for convenience been carried out by the addition of DP to aqueous solutions or suspensions of cell constituents. The pathology of DP poisoning is the same as that of CG.⁴⁷ The data on the toxicity of DP indicate that the $L(Ct)_{50}$'s do not differ markedly from those for CG²⁴ but the determinations are not adequate for a close differentiation. For the mouse a recent determination (140 animals) gives a 10-minute $L(Ct)_{50}$ of 3,600 mg min/m³ for DP³ to be compared with values of 1,800 and of 3,800 for CG (Table 1).

3.6 CARBONYL CHLOROFLUORIDE

The fluorine analog of CG, carbonyl fluoride, is of a low order of toxicity compared with CG³⁷ and has not been considered as a potential war gas. The compound in which only one of the chlorines is replaced by fluorine, however, has merited special study.^c

Carbonyl chlorofluoride has been prepared in

^c Additional compounds related to CG but of minor interest are included in Chapter 11.

yields approximating 25 per cent by heating CG with a tenfold excess of anhydrous hydrogen fluoride at 125–145 C in a pressure vessel. Small amounts of antimony pentafluoride promote the reaction and with this catalyst lower temperatures can be used. The products of the reaction are hydrogen chloride, carbonyl fluoride, and carbonyl chlorofluoride, the last of which can be separated by distillation.⁵ It has also been prepared in low yield by passing CG over calcium fluoride at temperatures from 150 to 325 C and at pressures from 7 to 79 cm of mercury. From the amount of uncondensed gas produced it appears that the ratio of carbonyl chlorofluoride to carbonyl fluoride produced is greater than 1 at temperatures below 270 C. The maximum conversion (6 per cent) was obtained at 260 C.¹¹

Carbonyl chlorofluoride is a gas boiling at –42 C. Its melting point is –138 C.⁵ Its odor closely resembles that of phosgene, various observers being in disagreement as to whether the two are distinguishable by odor.^{5,8} It reacts readily with solid sodium hydroxide or with soda lime, shows no tendency to etch glass,⁵ and undergoes no loss in toxicity on storage in copper for 13 months at ordinary temperatures,⁸ although storage in a tank lined with polymerized shellac led to decomposition.⁶

The protection against carbonyl chlorofluoride afforded by whetlerite charcoal is of the same order as that against CG.⁶

For most species the toxicity of carbonyl chlorofluoride is very close to that of CG. For the mouse the 10-minute $L(Ct)_{50}$ (220 animals) is 1,200 mg min/m³.⁸ compared with 1,800 for CG in determinations on the same strain of mice by the same laboratory. For the rat, guinea pig, and dog approximate determinations give 10-minute values of <2,700, <2,700, and <6,000⁸ to be compared with CG values of 1,400 (30 minutes), 1,300–2,200 (30 minutes), and 4,200 (20 minutes). CG is apparently much more toxic than carbonyl chlorofluoride for the rabbit, for which the $L(Ct)_{50}$ of CG is <1,000 (30 minutes) and that of the fluorine compound is approximately 7,000. Carbonyl chlorofluoride yields a symptomatology and pathology corresponding to that following phosgene poisoning.⁸

Any advantage which carbonyl chlorofluoride might have over CG would rest upon its lower boiling point, which might make it more effective in the production of crash concentrations. However, at the present time the procedures for its preparation are not satisfactory for large-scale work.⁸

Chapter 4

DISULFUR DECAFLUORIDE^a

By *Birdsey Renshaw* and *Marshall Gates*

4.1 INTRODUCTION

DISULFUR DECAFLUORIDE (S_2F_{10}) is a dense, highly volatile liquid whose comparatively odorless and nonirritating vapor is a lung-injurant similar in mode of action to phosgene; for some species it is at least as toxic as phosgene. It thus presents attractive features as a potential nonpersistent agent, and its synthesis has been carefully investigated by Canadian researchers, by NDRC Division 10, and by the Chemical Warfare Service. The synthetic methods developed to date require the use of elemental fluorine and give maximum yields of only about 30 per cent. The consequent difficulty and expense of its production in quantity at the present time have precluded serious consideration of its adoption as a standard agent.

The stability and other physical and chemical properties of disulfur decafluoride appear well suited to its effective dispersal from chemical munitions. The gas mask canister can be expected to protect against it approximately as well as against phosgene. Consequently, if its large-scale production were to become feasible in the future, its merits as a nonpersistent agent would be determined in large part by its toxicological properties, in particular by its toxicity for man, and by whatever advantages would accrue to a nonpersistent agent which, at least in the pure state and at moderate concentrations, is odorless and nonirritating. At present there are available no data upon which might be based an estimate of the incapacitating and lethal doses for man. If, as is the case for several of the smaller mammalian species, disulfur decafluoride should prove to be as toxic as or somewhat more toxic than phosgene, it would have some advantages over currently standardized nonpersistent agents. If, as appears to hold for the monkey, it should prove to be only one-tenth as toxic as phosgene, its large-scale use in warfare could hardly merit consideration.

^a Based on information available to Division 9 of the National Defense Research Committee [NDRC] as of August 1, 1945.

4.2 SYNTHESIS AND PROPERTIES^b

4.2.1 Synthesis

Disulfur decafluoride was discovered in 1934 by Denbigh and Whytlaw-Gray.³² It occurs as a by-product formed in small quantities (1 per cent) during the synthesis of sulfur hexafluoride from sulfur and elemental fluorine. In spite of extensive studies during World War II, its preparation in quantity remains difficult and expensive;^{12,25,29} it has not yet been possible to prepare it except by the use of elemental fluorine^c or to increase the yield based on fluorine above 30 to 34 per cent in spite of a thorough investigation of the reaction conditions.^{1,2,9,12,25,26,35d,g} The procedures which have given the best yields utilize the reaction of elemental fluorine, either pure or diluted with 5 to 30 parts of nitrogen, with solid roll sulfur, either pure or diluted with potassium or sodium fluoride. Adequate cooling of the reaction vessel is essential and either the fluorine or the sulfur must be diluted; oxygen and moisture must be excluded. Failure has attended attempts to convert sulfur hexafluoride, the principal product of the reaction, to disulfur decafluoride by a variety of methods which include its passage through an electric arc and its reaction with hydrogen sulfide or with molten potassium.^{35a}

4.2.2 Physical and Chemical Properties

Disulfur decafluoride is a colorless, mobile liquid boiling at 30.1 C; it solidifies at low temperatures and melts at -53 C.¹ Its liquid density is 2.00 at 20 C, its vapor density approximately nine times that of air.^{12,35f} The vapor pressure, which has been precisely determined as a function of temperature, is 235 mm at 0 C and 675 mm at 25 C.^{10,12,35d} It is virtually insoluble in water (<0.005 per cent by

^b For a more complete review the reader is referred to the Summary Technical Report of NDRC Division 10.

^c The production of elemental fluorine has been the subject of intensive investigation by several NDRC groups. It now appears possible to produce it in quantity at relatively low cost. The reader may consult the Summary Technical Report of NDRC Division 10 for a review of this work.

weight), in 0.9 per cent sodium chloride, and in 0.1M phosphate buffer at pH 7.4, but soluble in various common organic solvents;^{2,15,17} it is somewhat soluble in olive oil, with which it reacts to a limited extent.²

The thermodynamic properties of disulfur decafluoride have been evaluated using both thermochemical data and statistical considerations, and from these the gas-phase equilibria of a number of possible reactions of the sulfur fluorides have been calculated.^{6,8,10,31}

Disulfur decafluoride does not react with such agents as strong alkalis or acids, phosphorus pentoxide, or common solvents; fluorine has no action upon it at temperatures up to its decomposition point (160–210 C), but chlorine attacks it to yield a slightly volatile liquid.^{12,35d}

On prolonged contact with aqueous solutions, disulfur decafluoride appears to produce acid, but this conclusion must be regarded as tentative because the tests were made with a commercial preparation which may have contained small quantities of hydrolyzable impurities.^{15,17}

On activated carbon, disulfur decafluoride is catalytically decomposed to sulfur hexafluoride and sulfur tetrafluoride, the latter presumably decomposing further to sulfur difluoride and sulfur hexafluoride; nearly one-half of the weight of the original material appears as the hexafluoride.^{2,12} Thermal decomposition, which is slow at 200 C but rapid at 300 C, appears to involve similar reactions and gives sulfur hexafluoride as the principal product.^{2,12}

The ability of disulfur decafluoride to act as an oxidizing agent is an important property which may be involved in its physiological mechanism of action (see the following section) and which has been utilized in the development of methods for its detection and analysis.

4.2.3 Detection and Analysis

The comparative inertness of disulfur decafluoride limits the number of methods available for detection and analysis. Its oxidizing ability and its decomposition on charcoal have been utilized.

A number of easily oxidizable substances, including several oxidation-reduction indicators, have been examined as detectors for disulfur decafluoride.^{7,37} Of these *p*-phenylenediamine and *bis*(*p*-dimethylaminophenyl)methane appear to be the most suitable. A quantitative colorimetric procedure using the former has been developed for the analysis of chamber air in toxicity determinations.¹⁹

Disulfur decafluoride may be detected in air in concentrations as low as 10 µg/l by passing the air through charcoal and then filtering a fluoride ion indicator (e.g., thorium or zirconium alizarin sulfonate) through the charcoal. For use in detector tubes, however, charcoal decomposition followed by recognition of fluoride ion by standard methods is less satisfactory than the use of oxidation-reduction indicators.^{7,11}

The reaction of disulfur decafluoride with either sodium or potassium iodide to produce iodine can be used for both qualitative and quantitative analyses.^{7,24,30a} The reaction is suitable for determinations of concentration in gassing chambers and for analyses of canister effluents. Acetone bubblers, preceded by alkali scrubbers to remove hydrolyzable sulfur fluorides, are customarily employed.

The detection and analysis of disulfur decafluoride are reviewed in more detail in Chapters 34 and 37.

4.2.4 Stability

Disulfur decafluoride reacts slightly with iron at 55 C, but the reaction is sharply terminated, possibly by the formation of a protective coating. There is no reason to believe that the material cannot be stored satisfactorily in mild steel containers.^{2,12}

That disulfur decafluoride possesses sufficient stability to be dispersed without decomposition from explosive munitions is suggested by the results of the one test for which data are available.²³ A 75-mm shell was exploded in a large chamber; subsequent chemical analyses and toxicological bioassays of the chamber air demonstrated that at least 82 per cent of the dispersed agent was recoverable as such.

4.2.5 Canister Penetration

The protection afforded by the modern gas mask canister against disulfur decafluoride is reviewed in detail^d and the conclusion reached that it is comparable to that afforded against phosgene. As an example, no disulfur decafluoride appeared in the effluent for more than 130 minutes when the United States M10A1 canister was tested in the Intermittent Flow Canister Testing Apparatus E2 against 5 to 6 mg/l of the agent.²⁴ However, small amounts of sulfur hexafluoride, which is odorless and relatively innocuous,^{5,11,13,16a} penetrate charcoals almost immediately, and subsequently odorous and irritating substances (sulfur dioxide, hydrogen fluoride, or

^d See the Summary Technical Report of NDRC Division 10.

thionyl fluoride) appear in the effluent in concentrations which progressively increase to attain intolerable and potentially dangerous levels before toxicologically significant amounts of disulfur decafluoride^e are passed.^{3,11,24,33,34} Addition of soda lime removes these decomposition products and thereby materially increases the dosage against which charcoal affords useful protection.^{3,11,24}

4.3

TOXICOLOGY

Disulfur decafluoride is to be viewed as a non-persistent lung-injuring producing casualties qualitatively similar in character and time course to those caused by the inhalation of phosgene. At the concentrations which have been tested, it does not produce lacrimation or skin irritation.^{15,17,22} In marked contrast with phosgene and cyanogen chloride, for which the median detectable concentrations are less than 0.01 mg/l,²⁰ pure samples are odorless and non-irritating to the respiratory tract when breathed briefly at concentrations of at least 0.2 mg/l.¹¹ Other observers have ascribed to presumably impure preparations an odor similar to that of sulfur dioxide,^{15,17,29,39} and on several occasions the sniffing of a commercially prepared sample, which had a sulfurous odor, was followed by the development of mild nasal irritation of several hours' duration.^{1,15,17} It remains to be determined whether impurities were responsible for the odor and irritation described in the latter observations, and whether or not the pure material is odorless at concentrations higher than those which have been tested.

4.3.1

Toxicity for Animals

Most of the available toxicity data for disulfur decafluoride are summarized in Table 1. Included for the sake of comparison are the most nearly comparable data for phosgene (see also Chapter 3). By and large, the data do not substantiate a currently prevalent impression that disulfur decafluoride is distinctly the more toxic. Although this impression is probably correct for the mouse, rat, and goat, particularly at short exposure times, the opposite relation

holds for the guinea pig and monkey. The discrepancy is striking in the case of the monkey; the data, unfortunately limited in number, suggest that for this species disulfur decafluoride is only about one-tenth as toxic as phosgene.

Information bearing upon the effect of exposure time on the $L(Ct)_{50}$ of disulfur decafluoride and upon its rate of detoxification in the body is scanty. The data (Table 1) indicate that the $L(Ct)_{50}$ of disulfur decafluoride does not vary significantly with exposure time over the range 1 to 30 minutes. Thus, this relatively odorless compound does not exhibit the increased $L(Ct)_{50}$'s at short (1-minute) exposures which characterize phosgene and which have been associated with an inhibition of respiration due to sensory irritation (see Chapter 3), and it does not appear to be detoxified at a rate comparable to that for hydrogen cyanide (see Chapter 2). On the other hand, the results of a limited number of experiments suggest that animals can tolerate two to four exposures at 24-hour intervals to vapor dosages each of which is of nearly lethal magnitude.¹¹

A striking feature of toxicity data for disulfur decafluoride is the narrow range of concentration between that causing no deaths and that producing 100 per cent mortality; with phosgene, on the other hand, the dose-mortality curve is spread widely on the dose axis.³² As a result the curves for disulfur decafluoride and phosgene may cross, the latter compound killing more animals at relatively low dosages, the former, more at higher dosages. In spite of the steepness of the dose-mortality curve, however, disulfur decafluoride in sublethal doses does produce pulmonary pathological changes which in man might be of clinical and military significance.^{35c}

4.3.2

Symptomatology

Animals utilized for toxicity determinations have, in general, been exposed to dosages of less than three times the $L(Ct)_{50}$ and to concentrations of less than 5 mg/l. Under these conditions no obvious changes in respiration or other indications of sensory irritation are apparent^{11,22,32} and the animals appear normal upon return to their cages;¹¹ practically all fatalities occur between 1 hour and 2 days after exposure, the majority occurring between 3 and 20 hours.^{11,22,27} There seems to be a fairly definite inverse correlation between dosage and time for death.^{11,27} In a typical case culminating in death after 3 to 6 hours, the animal begins to appear quiet and depressed after 1 to 2 hours, its respiration be-

^e Thus, an interesting situation, which might or might not have military significance, could conceivably arise during exposures to high dosages of disulfur decafluoride: Masked troops upon inhaling the irritating but not dangerous substances in the canister effluent might suppose their protection inadequate; upon removing their masks, they would be exposed to the much less odorous and irritating but vastly more toxic disulfur decafluoride.

TABLE 1. Toxicity of disulfur decafluoride.

Included are the most nearly comparable data for phosgene. Unless otherwise noted, the $L(Ct)_{50}$'s are based on nominal concentrations and 10- or 15-day observation periods.

Species	Exposure time (min)	Disulfur decafluoride				Phosgene			
		Estimated $L(Ct)_{50}$ (mg min/m ³)	Number of animals	Time of death	Reference	Estimated $L(Ct)_{50}$ (mg min/m ³)	Number of animals	Time of death	Reference
Mouse	1	2,200	160		16b	3,450	240	4 hr-3 days*	14
	10	1,960	160		16b	1,800	220		14
	1	1,400-2,000	40		11				
	10	1,340	150	1-48 hr	11				
	30	1,000-1,400	40		11				
	10	1,900	160	<24 hr	22	3,650†	109	83% in 2 days	21
	10	1,000	30	1½-20 hr	27				
	30	1,620‡	160		32	1,980‡	175		32
Rat	1	2,300	8		30b	6,500	24	≤40 hr	14
	10	2,000-3,000	30	1-16 hr	11				
	20					1,800 ± ‡	46		18
Guinea pig	1					2,800	28	3 hr-2 days	14
	10	6,000 ±	20	1 hr-9 days	11				
	10	4,000-6,000	15	4 hr-2 days	27	1,900†	...		27
Rabbit	1					7,500 ±	8	3 hr-4 days	14
	10	6,000 ±	10	5-18 hr	11				
	10	4,000-6,000	6	7-21 hr	27	13,000†	...		27
Cat	1					3,300	7	<1 day	14
	10	4,500 ±	10	50 min-12 hr	11				
	10	<4,000	4	6-8 hr	27	3,500†	...		27
Dog	1	5,000	16		16b	7,000	9	<1 day	14
	10	4,000-6,000	10	6-20 hr	11				
	20					4,200	20	6-20 hr	14
Goat	10	4,000-6,000	9	6 hr-15 days	27	8,500†	...		27
Monkey (Rhesus)	1					600-1,000	13	3-15 hr	14
	10	9,000 ±	10	4-5½ hr	16b	1,200	10		14, 36

* A few died after 3 to 10 days. † Analytical concentration. ‡ Two-day observation period.

coming shallow and rapid; after an additional 1 to 2 hours, respiration becomes labored; cyanosis then soon sets in, to be quickly followed by asphyxial convulsions and death accompanied by a flow of colorless foamy fluid from the nose and mouth.¹¹

In an experiment in which mice were exposed to a high concentration (45 mg/l), symptoms and death occurred with striking rapidity. The animals immediately flattened out on their bellies and began gasping for breath. All died within 6 to 11 minutes after the beginning of the 10-minute exposure.^{11,35b}

4.3.3 Pathology

Disulfur decafluoride acts primarily as a pulmonary irritant, producing an anoxic death due to massive, fulminating pulmonary edema and hyperemia. It differs from phosgene and chlorine in that it does not injure the columnar epithelium of

the bronchi and bronchioles, the pathological changes being confined to the alveoli and the pulmonary connective tissue, and being more prominent in the hilar than in the peripheral portions of the lung.¹¹ Some observers have also reported acute vesicular emphysema and, in a large proportion of animals, marked pleural effusion;²⁷ inasmuch as the pleura show no evidence of acute inflammation, it has been suggested that the pleural fluid arises from the lymphatics draining the edematous lungs.²⁷ In some instances marked edema of the mediastinum, accompanied by distention of the mediastinal lymphatics has been noted.²⁷

In comparison with phosgene, disulfur decafluoride produces in dogs a more fulminating lung edema and hemoconcentration but less marked blood pressure or other circulatory changes.^{19,35c} The hematocrit may rise slowly for some hours and then leap to high

values. Plasma protein concentration arises progressively. There is no initial bradycardia such as that which occurs with phosgene, and, in contrast to poisoning by the latter agent, the pulse rate does not become greatly accelerated in the late stages. Arterial and venous pressures are little altered. Respiration is increased more gradually than by phosgene but eventually rapid and shallow breathing is established. Arterial and venous oxygen concentrations fall slowly at first, and then rapidly to values incompatible with life.

No extrapulmonary pathological changes other than congestion and fatty degenerative changes in the liver and kidney — effects presumably secondary to lung edema — have been reported as sequelae of inhalation of the vapor;^{11,19,27,35e} in particular, the eyes, nasopharynx, trachea, and lymphoid tissue appear normal. Observations on the corneal circulation of gassed dogs revealed hemoconcentration but not the other changes (cell clumping, vessel spasm, and local transudation of fluid) which have been observed in phosgene poisoning.¹⁹ Dogs with one bronchus plugged during gassing and the other plugged subsequently to prevent edema fluid from pouring into the protected lung sometimes survived exposures to what would have been lethal dosages for normal dogs, and regularly lived longer than bilaterally gassed animals; the protected lung remained grossly and microscopically normal.¹⁹

4.3.4 Physiological Mechanism

The physiological mechanism of action of disulfur decafluoride has not been systematically investigated and definitive conclusions cannot be drawn at the present time. It is evident from the findings reviewed above that the significant pathological changes consequent upon inhalation of the vapor are confined to the pulmonary tissues. That other tissues are susceptible, however, and would be affected if sufficient quantities of the agent reached them, is indicated by the action on hemoglobin and by the lethal effects of intraperitoneal injections (see below).

In evaluating the significance of the following isolated findings bearing on mechanism, and in planning future studies, the following facts set forth in the chemical section above may be recalled: (1) disulfur decafluoride acts as an oxidizing agent, and (2) its carbon-catalyzed decomposition in the presence of moisture involves the formation of sulfur hexafluoride and other substances among which may be thionyl fluoride, hydrogen fluoride, and sulfur dioxide.

As in the case of water and salt solutions, addition with shaking of a plant-run sample of disulfur decafluoride to blood plasma resulted in the slow production of acid; the experiment has not been repeated with the purified compound.^{15,17} In the presence of the same plant-run sample, bromthymol blue in 0.01M phosphate buffer at pH 7.4 faded irreversibly in about 1 day; the rate of fading was accelerated in the presence of dissolved sodium chloride and in more alkaline solutions.^{15,17} It is not known whether this reaction was an oxidation of the indicator or merely an acceleration of the tendency of brominated sulfonphthaleins in aqueous solution to form the corresponding carbinols.

Upon shaking whole blood with the plant-run disulfur decafluoride, the hemoglobin slowly darkened with the evolution of gas bubbles. Addition of an acetone or ether solution of the agent produced these effects immediately. Solutions of oxyhemoglobin obtained by hemolyzing and filtering blood behaved as did whole blood.^{15,17} At pH 7.4 the absorption spectrum of the altered pigment resembled that of methemoglobin in general features but not in all details; over the wavelength band 370 to 700 m μ it had no resemblance to the spectrum of oxyhemoglobin treated with sodium fluoride.

Rapid local damage and death with hemoconcentration follow the intraperitoneal injection of disulfur decafluoride as liquid or vapor.^{19,38} In one experiment with a rat injected with the gas, death occurred with extreme hemoconcentration after a latency of 2 hours; at autopsy the lungs were clear but the peritoneal cavity contained 3 cc of fluid; injection of this fluid into a second rat had no detectable effects.³⁸

A single analysis of the pleural fluid accumulating after lethal gassing with disulfur decafluoride revealed a fluorine content of 2 mg/100 ml.^{35c}

Traces of sulfur hexafluoride were obtained from the reactions of disulfur decafluoride with olive oil and with pieces of excised rat lung;¹² the reactions were sharply limited in extent.

4.3.5 Prophylaxis and Therapy

The results of a single exploratory study¹⁹ indicate that prophylactic inhalation of magnesium carbonate dusts and intramuscular injection of magnesium sulfate solutions may have limited value in prolonging and saving the lives of mice gassed with disulfur decafluoride. Intraperitoneal injection of calcium chloride following exposure appeared to be harmful

TABLE 2. Physical properties of disulfur decafluoride and of currently standardized nonpersistent agents.

Property	Disulfur decafluoride	Phosgene	Hydrogen cyanide	Cyanogen chloride
Liquid density (g/ml at 25 C)	2.0	1.36	0.68	1.2
Vapor density (air = 1)	8.1	3.4	0.93	2.0
Boiling point, C	30.1	8.3	26	12.6
Melting point, C	-53	-104	-13.4	-7
Latent heat of evaporation, cal/g	25	60	210	135
Vapor pressure, mm Hg				
at 25 C	675	1,400	740	1,200
at -20 C	87	230	88	180
Volatility, mg/l				
at 25 C	9,000		1,060	
at -20 C	1,400	1,460	145	680

and the inhalation of calcium carbonate dusts prior to exposure was without clear effect. Prophylactic injections of hexamethylenetetramine, a chemical specific for phosgene, were without effect, and 2,3-dimercaptopropanol (BAL), a similar specific for arsenic and cadmium, was detrimental. Prophylactic intramuscular injections of pitressin did not alter the course of the poisoning; exercise immediately subsequent to exposure was not harmful.

4.4 EVALUATION AS A WAR GAS

Because of the difficulty and expense of manufacture on a large scale at the present time, it has not been practicable to make disulfur decafluoride for use in World War II. The available information does not permit a clear decision as to whether it would possess greater general utility than currently standardized agents if its production and use in quantity were to become feasible. At the present time it should be evaluated in comparison with phosgene, the standard agent to which it is most similar in physical and toxicological properties. In terms of current concepts of chemical warfare, the tentative conclusion seems justified that its use on the battlefield would not demonstrate it to be *markedly* superior to phosgene as a casualty-producing agent and might reveal it to be definitely inferior.

The physical properties of disulfur decafluoride are well suited to its dispersion in high concentrations as a nonpersistent agent (Table 2). Moreover its high liquid density would permit significantly greater amounts to be carried in any given munition than is possible with phosgene, cyanogen chloride, or hydro-

gen cyanide. So far as is known, its stability would suffice to permit its storage in currently available chemical munitions and its dispersal from them without destruction. Its insolubility in water and resistance to hydrolysis would give it an advantage over phosgene for use under those conditions of terrain and meteorology which permit clouds of "non-persistent" gases to exist for many minutes.

The protection afforded by modern gas mask canisters against disulfur decafluoride, like that afforded against phosgene, is so good that with reasonable munitions expenditures one could not hope to set up dosages sufficiently large to break the canister, except under very special circumstances. One would therefore expect that the bulk of the casualties to be realized from its use would be among individuals who, because of lack of time, unawareness of the presence of the poison, or other reasons, would be exposed unmasked or imperfectly masked. Consequently, attention focuses on toxicological properties.

To whatever the extent that relative lack of odor and of irritating properties are desirable in a non-persistent agent, disulfur decafluoride (at least in the pure state) has an advantage over phosgene and cyanogen chloride. However, the critical toxicological data, namely the incapacitating and lethal dosages for man, are not available. If, as holds true for some animal species, it is as toxic or more toxic than phosgene, its use (assuming availability) in place of this agent would merit consideration. If, on the other hand, it is only one-tenth as toxic as phosgene (as appears to be the case for the Rhesus monkey), its utility as an offensive agent would hardly merit its production for use in warfare.

Chapter 5

MUSTARD GAS AND OTHER SULFUR MUSTARDS^a

By *Marshall Gates and Stanford Moore*

5.1

INTRODUCTION

A MAJOR PART of the activities of Division 9 of the National Defense Research Committee [NDRC] centered around the defensive and offensive problems presented by mustard gas and closely related vesicant agents. Chapters in Parts III, IV, and V deal in detail with the mechanism of action of agents in this class and means for protection and detection. The present chapter deals mainly with the methods for preparation of mustard gas and its analogs, a tabulation of the compounds which have been prepared, and the basic toxicological measurements on the more important members of the series.

Mustard gas (H) was the principal battle gas of the last year of World War I. H was the agent which was manufactured and stocked in the largest tonnage for possible use in World War II. The more recent investigations on the subject of H have greatly extended the knowledge of the mechanism of action of the agent, the information on its behavior in munitions under field conditions,¹⁷³ and the means for protection against the vesicant action of the vapor and liquid forms of the agent.

Two relatively nonvolatile vesicant agents have been studied in detail for possible use in mixtures with H. They are 1,2-*bis*(β -chloroethylthio)ethane (Q) and *bis*(β -chloroethylthioethyl) ether (T). These two agents have a higher vesicancy in contact with bare skin and greater persistence on terrain than H, but because of their low vapor pressure they lack the ability to produce casualties by vapor action.

HQ and HT mixtures have been prepared on a small scale. For special purposes the nitrogen mustards would have some uses (see Chapter 6). Among the hundreds of compounds that have been studied in the sulfur mustard series since H was first used in 1917, no agent has been found to have a more advantageous combination of toxicological, chemical, and physical properties than H.

^a Based on information available to NDRC Division 9 as of Jan. 1, 1946.

5.2 PRODUCTION PROCESSES FOR H, HQ, AND HT

5.2.1

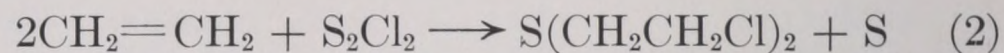
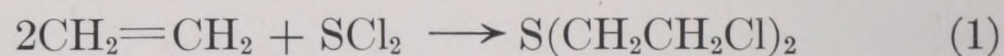
General Methods

The original laboratory methods of Guthrie²⁷⁰ and of V. Meyer²⁷³ for the preparation of H^b were both put to use on an industrial scale during World War I.

The Meyer process, applied to large-scale production by the German Dye Trust, consisted essentially of the chlorination of thiodiglycol by hydrochloric acid. The thiodiglycol required was prepared from ethylene chlorohydrin by the action of sodium sulfide.²⁷⁸

The English had begun erection of a plant for the manufacture of mustard gas from thiodiglycol and thionyl chloride in 1918, but apparently the plant did not come into production before the end of World War I. Other chlorinating agents, such as phosphorus trichloride and thionyl chloride, have been used, and other processes, notably the action of hydrogen sulfide on ethylene oxide, are now available for the preparation of thiodiglycol.

The Guthrie process involves the interaction of ethylene and sulfur chlorides and has been formulated as follows:



although the course of the second reaction is more complex than required by this equation. Various modifications of this process provided all the mustard gas used by the Allies in World War I, the three principal processes being:¹²

1. *The French process* (Cattelain process). In this process a 10 per cent solution of sulfur dichloride in carbon tetrachloride was saturated with ethylene, and the dilute solution of mustard gas so obtained was stripped to a concentration of about 85 per cent.

2. *The 60 C process*. Dry ethylene was led into sulfur monochloride maintained at 55–60 C. Under these conditions about one-half of the excess sulfur

^b Both Despretz²⁶⁷ and Riche²⁷⁴ appear to have prepared *bis*(β -chloroethyl) sulfide before Guthrie.

remained in solution as polysulfides, whereas the other half separated on standing or on treatment with moist ammonia.

3. *The Levinstein process.* In this process, also known as the "30 C process," pure ethylene was led into a mixture of sulfur monochloride and crude mustard gas maintained between 30–34 C. Under these conditions the precipitation of sulfur was minimized and charging operations were facilitated. This process had been investigated by Pope and his co-workers²⁷² and was successfully used on a plant scale by the British firm of Levinstein, Ltd., and by the United States Chemical Warfare Service [CWS].

The thiodiglycol method for the preparation of pure H was not used by the United States or Great Britain during World War II, although successful laboratory procedures for carrying out the synthesis by both batch and continuous processes have been worked out.^{8,27,40} Apparently the Germans, as in 1917–18, relied principally on this method for their mustard stocks, although the thiodiglycol was obtained by the action of hydrogen sulfide, synthesized catalytically from hydrogen and sulfur vapor, on ethylene oxide, and the hydrogen chloride was obtained by burning hydrogen and chlorine.^{172a,246}

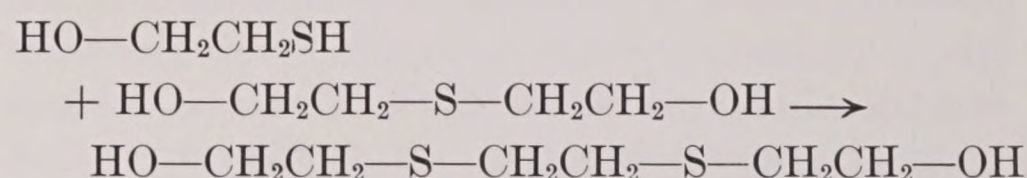
Although the thiodiglycol process has not been used by the Allies to produce H, a modification of it has been used to produce HT based upon the Oxford and Davies'²⁶⁵ discovery that the incomplete chlorination of thiodiglycol produces T in addition to H. This reaction has been used on a plant scale to produce HT, which is a mixture of these two materials with smaller amounts of high molecular weight substances.²³⁵ The process is carried out essentially as follows: thiodiglycol, preheated to 60 C and mixed with concentrated hydrochloric acid, is treated for 1 hour with hydrogen chloride in a tile-lined vessel. During the treatment the temperature rises to 110–115 C. Variation of the reaction conditions, especially the temperature, allows some variation in the composition of the product. Normally, a mixture of 60 per cent H and 40 per cent T (nominal) is obtained. The 40 per cent T contains homologs of T and related compounds, including a small amount of 1,2-bis(β -chloroethylthio)ethane (Q) formed from impurities present in crude thiodiglycol. This mixture melts at about 0 C, and has excellent stability. It is more vesicant than H against bare skin. Two plants each capable of producing 50 tons per week of HT 60/40 were erected in England.^{108,235}

The process has also received study under a CWS

contract at the Monsanto Chemical Company, and a continuous process consisting essentially of counter-current passage of thiodiglycol and hydrogen chloride through a packed column has been developed. Superheated steam is used both as a source of heat and as a means of steam-distilling off the H formed so that subsequent reblending can be made to produce HT of any desired composition. A pilot plant for the process has been designed.¹⁷⁰

Pure T can be prepared through T glycol¹ by the action of thionyl chloride¹⁰⁵ or by the photochemical addition of bis(β -mercaptoethyl) ether to vinyl chloride,^{59,131} as described later in this section.

During the course of an investigation of the French process for the continuous conversion of thiodiglycol into H, the British in 1939 isolated Q, known since 1921, in small amounts from the product. Its formation was shown to be due to an impurity, β -mercaptoethanol, in the thiodiglycol used. Under the conditions of the reaction, this condenses with thiodiglycol to give Q glycol which is then transformed into Q.



The reaction has been investigated by the British using different proportions of β -mercaptoethanol and thiodiglycol under a variety of conditions, and has led to the so-called HQ process, in which a mixture of thiodiglycol and β -mercaptoethanol containing about 15 per cent of the latter is added to an excess of concentrated hydrochloric acid at 80 C.^{77,213,214,235} The product contains 25–30 per cent Q, together with a small amount of bis(β -(β -chloroethylthio)-ethyl) sulfide, and melts at about 5 C. Larger amounts of the latter substance are produced if the content of β -mercaptoethanol of the charge is higher than 15 per cent. The process has been worked out only on a laboratory scale, but is considered capable of being carried out in the HT plants. The product is somewhat more vesicant than HT against bare skin.

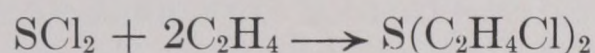
The HQ process has also been investigated under a CWS contract by the Monsanto Chemical Company, whose conclusions are in substantial agreement with those of the British, except that optimum results were obtained by gassing with hydrogen chloride a 10 per cent β -mercaptoethanol in thiodiglycol solution previously mixed with hydrochloric acid.

The process developed was considered suitable for scaling up in existing L plants.¹⁷⁰

Pure Q can be prepared by the photochemical addition of 1,2-ethanedithiol to vinyl chloride, as described in Section 5.2.7.

5.2.2 Sulfur Dichloride Processes

Ethylene and sulfur dichloride react according to the equation:



and processes employing sulfur dichloride are not attended by the sulfur precipitation or the polysulfide formation characteristic of sulfur monochloride processes, which are now considered obsolete by the British. The reaction is much more rapid than that between ethylene and sulfur monochloride, and adequate cooling is required.

Sulfur dichloride exists at ordinary temperatures as an equilibrium mixture with sulfur monochloride and chlorine, approximately in the proportion 85/10/5. The equilibrium is mobile at ordinary temperatures and it is consequently not possible to obtain pure sulfur dichloride by simple fractionation at atmospheric pressures, although at low temperatures the rate of attainment of equilibrium is slow enough for fractionation at reduced pressures to be effective. The discovery by British investigators that the presence of phosphorus pentachloride markedly decreases the rate of attainment of equilibrium, however, has allowed the preparation in quantity of pure sulfur dichloride (99.5 per cent) by fractionation of the equilibrium mixture at atmospheric pressure in glass apparatus. Metals in contact with the dichloride promote dissociation to a variable degree. Brass, one of the least active, when used in still construction, allows production of sulfur dichloride of 98–98.5 per cent purity.²³⁵

The type of sulfur dichloride used affords a basis for classification of the various British H processes. Thus:

1. HS (obsolete). In this process a 1/6 mixture of crude (equilibrium mixture) sulfur dichloride and carbon tetrachloride were treated with ethylene at 25 C. The process was continuous and the product was obtained by stripping off the carbon tetrachloride under reduced pressure to a content of about 15 per cent, this being sufficient to reduce the melting point of the mixture to less than 5 C.

2. HM and HB. HS produced by a modification of the procedure just described is stripped under 70–

100 mm to a carbon tetrachloride content below 1 per cent and is then diluted with 7–10 per cent monochlorobenzene or benzene to form HM or HB. These have better pressure stability than HS, chiefly because of the thermal decomposition of unstable factors (principally trichloromustard) during stripping.

3. HMD, HBD, and HCD. These processes utilize pure sulfur dichloride, prepared by continuous two-stage distillation of sulfur dichloride stabilized by phosphorus pentachloride. In the plant, the first distillation stage is carried out in a cupronickel column to separate chlorine and sulfur dichloride from sulfur monochloride, and the second stage, which separates sulfur dichloride from chlorine, is carried out in glass. The continuous HMD reaction takes place in nickel reactors with only enough solvent (monochlorobenzene) present to give the required freezing point to the product, which requires no stripping. The reaction has been run with benzene and carbon tetrachloride as solvents, giving HBD and HCD.

A similar process for two-stage distillation of stabilized sulfur dichloride using brass columns has been developed, and sulfur dichloride produced in this way and containing 1–1.5 per cent sulfur monochloride gives HMD/B and HBD/B when used in the H processes. Further identifying letters signify the type of reactor used (nickel or cast iron).²³⁵ Heating for short periods (1/2–1 hour) at 165–180 C imparts greatly improved pressure stability to HMD and HBD.²³⁵

Apparently the Germans had also built plants for the continuous production of sulfur dichloride mustard.^{172a}

5.2.3 Sulfur Monochloride Processes

1. *South African (DESA) process.* In this process controlled precipitation of the excess sulfur is achieved by using ethylene saturated with alcohol vapor. The gas is passed into a batch of sulfur monochloride held at 35 C and is recirculated after washing and drying by brine cooling. A batch of 1,200 lb of sulfur monochloride requires 12 hours for reaction. After removal of the precipitated sulfur in a settler the H layer is stripped of a low-boiling fraction and then distilled at approximately 35 mm from a mild steel pot and condensed in lead. The pressure stability of the product is good.²³⁵

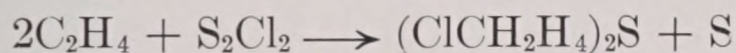
2. *The CWS Levinstein process.* This process has been standard with the American Chemical Warfare

Service since World War I, and extensive studies on the stabilization, storage stability, purification, composition, and behavior in the field of Levinstein mustard have been carried out in this country.

Sulfur monochloride is added to a seed charge of H in mild steel reactors to give a concentration of about 25 per cent. Ethylene is then passed in and further monochloride is added to maintain its concentration between 18 and 22 per cent. On completion of the addition, ethylene is passed in until the sulfur monochloride content falls to less than 0.05 per cent. The excess sulfur is largely retained in solution in the form of polysulfides. Nine hours are required to complete a 6-ton batch. Brine cooling is necessary during the early part of the reaction to maintain the temperature at 35 C.

5.2.4 The Composition of Levinstein H

Most of the early work on Levinstein H or on sulfur monochloride H, since many of the early experiments were carried out on "60 C mustard," was concerned with accounting for the excess sulfur required by the equation



Among the theories proposed to explain the failure of this sulfur to precipitate completely were the following:

1. The excess sulfur is present in the mustard in colloidal solution — "pseudo-solution."^{264,272} In support of this, the fact that the sulfur in 60 C H could be largely precipitated by heating to 100 C without changing the freezing point of the H was brought forward. Likewise, the precipitation of sulfur from 30 C H on dilution with alcohol appeared to support this hypothesis.

2. The sulfur is present as the dispersed phase of a two-phase liquid-liquid dispersion.²⁶² The difference in properties of various Levinstein samples was held to be due to differences in the degree of dispersion. The precipitation of sulfur by the addition of ether without change in freezing point was attributed to the removal of the dispersed phase. Attempts to prepare stable dispersions, however, failed.

3. The sulfur is present in the form of a loose compound with mustard itself.²⁶⁹ As evidence for this point of view the insolubility of sulfur in H, together with the fact that sulfur dissolved in sulfur monochloride H by heating precipitates quantitatively on cooling, was presented. The sulfur cannot be combined with *bis*(β-chloroethyl) sulfide itself, how-

ever, unless the complex is dissociable at room temperature.²⁷²

Other early evidence for compound formation (not necessarily with H itself) was the production of sulfuric acid by oxidation of industrial H²⁶⁶ and of sulfuric acid and a chlorine-containing alkane sulfonic acid by oxidation of a distillation residue of the approximate composition $(\text{ClCH}_2\text{CH}_2)_2\text{S}_5$ from 60 C H.²⁶⁴ A solid substance of the composition $(\text{ClCH}_2\text{CH}_2)_2\text{S}_3$ which could be oxidized to sulfuric acid and 2-chloroethanesulfonic acid was isolated by fractional distillation of 60 C H and it was shown that its formation was favored by lower temperature reaction of ethylene and sulfur monochloride.²⁷²

4. The sulfur is present partly in combination, partly as a colloidal dispersion or solution.²⁶⁸ This hypothesis was based upon the fact that only a part of the excess sulfur can be precipitated by heating, freezing, or treatment with moist ammonia.

In a series of distillation studies on Levinstein mustard of current manufacture carried out at Edgewood Arsenal,¹⁰⁰ the distillate obtained by the CWS specification assay for Levinstein H was subjected to fractional distillation and found to consist of pure *bis*(β-chloroethyl) sulfide (78 per cent) and a residue (20 per cent). Fractionation of the residue gave about 35 per cent of *bis*(β-chloroethyl) sulfide, 28 per cent of *bis*(β-chloroethyl) disulfide (HS_2), and 20 per cent of residue, with about 15 per cent loss during the distillation.

In order to minimize changes in composition occurring during distillation by ordinary methods, molecular distillation was resorted to. A preliminary distillation gave three fractions, the first of which was subsequently fractionated into three relatively volatile components, possibly chlorinated hydrocarbons, which contained no sulfur. The next two consisted essentially of *bis*(β-chloroethyl) sulfide. The residue was rich in sulfur, containing 4.5 molecular proportions of sulfur to every 1 of chlorine.

Repeated passage of crude Levinstein H through the molecular still at successively higher temperatures gave *bis*(β-chloroethyl) sulfide fractions progressively richer in sulfur. This increase was attributed to the presence of increasing amounts of HS_2 in the distillate. The unstable residue from these distillations could be separated into two components, an acetone-insoluble fraction having a composition corresponding approximately to $(\text{ClCH}_2\text{CH}_2)_2\text{S}_{12}$, and an acetone-soluble fraction of composition corresponding to $(\text{ClCH}_2\text{CH}_2)_2\text{S}_{4.5}$. The acetone-insolu-

ble residue deposited sulfur when allowed to stand. Treatment with gaseous ammonia caused rapid precipitation of sulfur, and the material remaining was found to have a composition corresponding closely to $(\text{ClCH}_2\text{CH}_2)_2\text{S}_5$.

Only slightly different results were obtained in similar distillation studies on Levinstein of 1937 manufacture and on Levinstein of 1918 manufacture.

From these results and those of other investigators it was postulated that the chief impurities in Levinstein mustard were polysulfides of variable composition. The transient existence of a polysulfide of any definite composition was attributed to the probable ability of the $-\text{CH}_2\text{SSCH}_2-$ linkage easily to gain or lose sulfur atoms. It did not appear possible to distill Levinstein H without altering its composition.

A review of the evidence available at the time of this work indicated that Levinstein mustard was composed of:

1. Gases, noncondensable at -78°C ; probably ethylene.
2. Chlorinated hydrocarbons; 1 per cent or less.
3. β -Chloroethyl β -chlorovinyl sulfide as such or as trichlorodiethyl sulfide.
4. *bis*(β -Chloroethyl) sulfide; 60 to 70 per cent.
5. *bis*(β -Chloroethyl) disulfide; free and as polysulfides.
6. Diethylene disulfide, partly free as monomer (dithiane) and polymer, and partly potential.
7. Sulfur, free and as polysulfide.

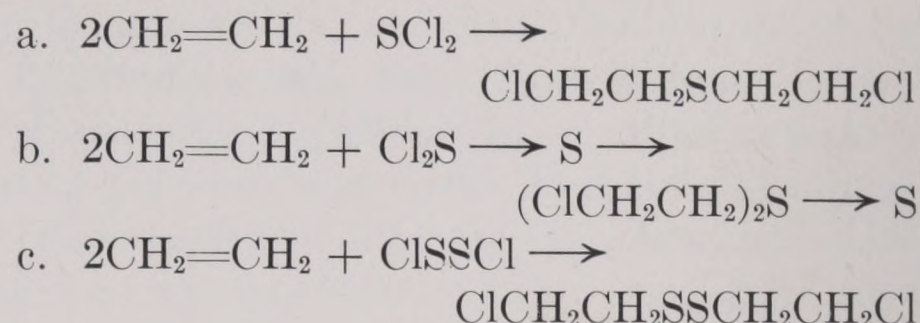
The results of some experiments on methanol extraction of Levinstein H also led to the conclusion that polysulfides are present.²⁴⁹ Cold methanol extraction of a sample of American Levinstein H left an insoluble residue of low vesicancy with the approximate composition $(\text{ClCH}_2\text{CH}_2)_2\text{S}_9$. The soluble portion, after stripping of solvent and removal of most of the H by freezing, was again fractionated by methanol extraction, yielding an insoluble fraction of the approximate composition $(\text{ClCH}_2\text{CH}_2)_2\text{S}_8$ and a soluble fraction whose composition approached $(\text{ClCH}_2\text{CH}_2)_2\text{S}_3$ but which contained about 10 per cent H. In view of the now known lability of the higher β -chloroethyl polysulfides, however, it may be unsafe to assume that material which has been stripped of methanol by distillation is unaltered.

In 1943 a theory of the formation and composition of Levinstein H which explained many of the available data was proposed by workers in the CWS. The salient points of this theory were the following:¹¹⁸

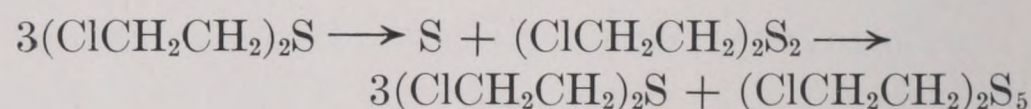
1. Sulfur monochloride was supposed to be an

equilibrium mixture of at least the following components: sulfur in solution, sulfur dichloride, and two isomeric forms of sulfur monochloride, ClSSCl and $\text{Cl}_2\text{S} \rightarrow \text{S}$.

2. Ethylene may react with all of these except sulfur.

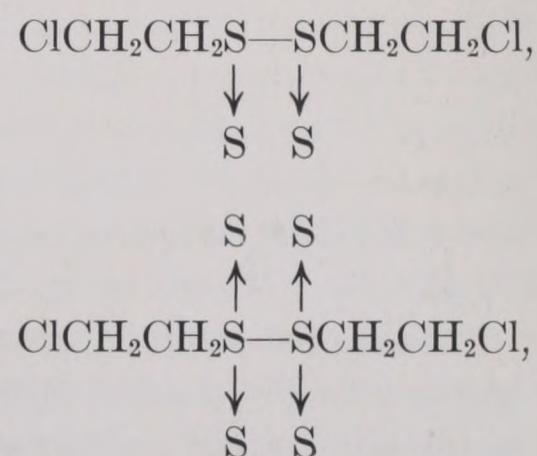


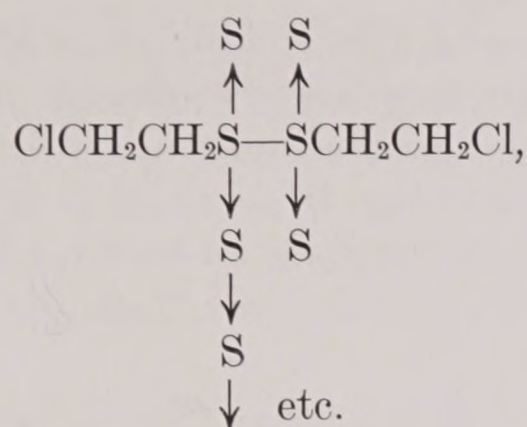
Since the compound $(\text{ClCH}_2\text{CH}_2)_2\text{S} \rightarrow \text{S}$ had not been isolated, it was postulated that it could have only a transient existence, decomposing into *bis*(β -chloroethyl) sulfide and sulfur. This "nascent sulfur" could then be taken up by the disulfide, $\text{ClCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{Cl}$, to form higher polysulfides. The number of sulfur atoms in the polysulfide would depend upon the proportions in which the two forms of the disulfide were present. For example, if the ratio were three to one, the resulting mixture would contain 62 per cent by weight of *bis*(β -chloroethyl) sulfide, and the polysulfide would be a pentasulfide.



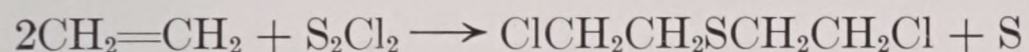
It was suggested that the ratio of $\text{Cl}_2\text{S} \rightarrow \text{S}$ to ClSSCl might be changed by altering conditions so as to increase the amount of $\text{Cl}_2\text{S} \rightarrow \text{S}$, which in turn would increase the amount of *bis*(β -chloroethyl) sulfide and sulfur formed. This would account for the higher yield of *bis*(β -chloroethyl) sulfide and for the precipitation of sulfur actually observed when the reaction is run at 60°C .

Available analytical data on the sulfur-chlorine ratio in Levinstein mustard and on the relation between freezing point and *bis*(β -chloroethyl) sulfide content of Levinstein mustard were used to extend and support the hypothesis. It was postulated that the principal impurities in Levinstein had the structures

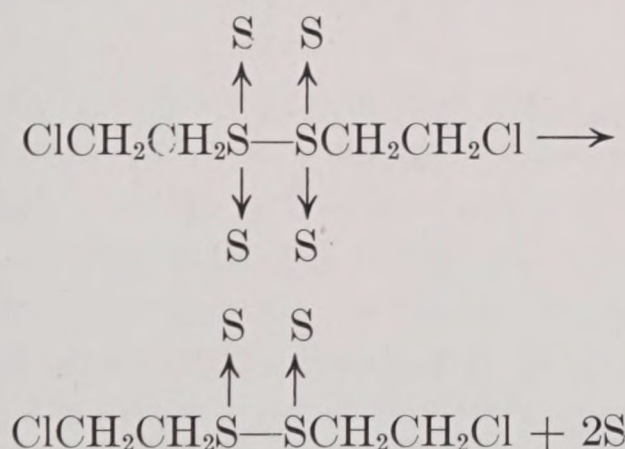




Excess sulfur could be stripped from impurities of this type without altering the mole fraction of impurity and consequently the melting point should remain constant, as is actually observed. The *bis*(β -chloroethyl) sulfide content of Levinstein H as determined by distillation and as calculated from its freezing point was shown to be very nearly the same on the assumption that the molecular weight of the impurity is 319, corresponding to *bis*(β -chloroethyl) hexasulfide (HS_6). If the equation



actually represents the Levinstein reaction, then it would appear that one atom of sulfur per molecule of H or about 16.8 per cent of the total product should be precipitated. Actually it is possible to induce only about half of this amount of sulfur to precipitate from Levinstein mustard. Analyses of fresh Levinstein H show that it contains approximately 37 per cent chlorine and 33 per cent sulfur, giving a sulfur-chlorine ratio of 1/1. It was postulated by the CWS workers that the 30 per cent impurity in Levinstein mustard was HS_6 , leading to a chlorine and sulfur content in the mixture of 37.9 per cent and 32.2 per cent, respectively. During aging or stripping HS_6 was assumed to lose sulfur until the more stable level HS_4 was reached.



This would represent a loss of sulfur amounting to 6 per cent of the total weight of the product. It would have no effect on the mole fraction or on the freezing point but would raise the weight-fraction of *bis*(β -chloroethyl) sulfide to about 0.75, with about 25 per cent of polysulfides still present. This mixture would

contain 27.7 per cent sulfur and 40.4 per cent chlorine, which is in close agreement with the observed values for aged or stripped Levinstein mustard.³⁵

This hypothesis, which has become known as the Reid-Macy hypothesis, as to the formation of and the composition of Levinstein mustard, was a significant forward step, but it was based on scanty experimental evidence and although substantially correct as an overall view, required considerable modification in the light of later experimental data.¹²⁷

The NDRC Division 9 group which undertook a study of Levinstein mustard in May 1943³⁵ introduced a valuable technique for the quantitative removal of *bis*(β -chloroethyl) sulfide from Levinstein H without altering the composition of the polysulfide fraction. This removal was achieved merely by exhaustive hydrolysis,³⁵ and its success depends upon the fact that the polysulfides in Levinstein are stable toward water at room temperature, whereas *bis*(β -chloroethyl) sulfide is readily hydrolyzed. The progress of the hydrolysis is followed by titration of the hydrogen or chloride ion produced. When the rate of hydrolysis becomes negligibly small, the non-hydrolyzed residue amounts to about 30 per cent by weight of the original product. The composition of the dark, oily residues from different samples of Levinstein mustard is not the same but varies between values corresponding to $(\text{ClCH}_2\text{CH}_2)_2\text{S}_6$ (HS_6) and $(\text{ClCH}_2\text{CH}_2)_2\text{S}_9$ (HS_9).

These residues deposit sulfur slowly. When treated with Cellosolve, in which the polysulfides are fairly soluble but in which sulfur has very limited solubility, sulfur is observed to separate in the form of fine crystals, and a small amount of dark gum remains insoluble. This gum has the composition of a high molecular weight polysulfide such as $(\text{ClCH}_2\text{CH}_2)_2\text{S}_{12}$. It invariably deposits sulfur within a few days and takes on the appearance of crystalline sulfur.

The soluble polysulfides can be recovered by washing the Cellosolve solution with water until the Cellosolve is completely removed, drying the resulting oil in ether solution, and removing the ether. A clear amber oil, containing slightly less sulfur and slightly more chlorine than a compound having the composition of *bis*(β -chloroethyl) pentasulfide, results.

A more complete characterization of these polysulfides was made possible by their synthesis. Oils having the characteristics of higher polysulfides were prepared by heating the known HS_3 , with excess sulfur under moderate conditions, by allowing it to stand in the presence of sulfur monochloride, or by

heating it with methyl tetra- or pentasulfides. When these oils are stripped of their excess sulfur by the Cellosolve treatment or by moist ammonia, they resemble the Cellosolve-stripped Levinstein residues of composition corresponding to HS_5 in appearance, odor, refractive index, density, polarographic behavior, and analysis. HS_5 was also prepared by heating HS_2 with excess sulfur under more stringent conditions, although sulfur monochloride and methyl polysulfides had no sulfurizing action on this disulfide. All of the synthetic polysulfides, if allowed to stand without being stripped with Cellosolve, deposit sulfur gradually until the composition of the residual oil approaches that of the pentasulfide. The solubility of sulfur in HS_5 proved to be about 7 per cent or 0.6 gram-atom so it is to be expected that sulfur would no longer be precipitated when the sulfur content of the polysulfide had decreased to 5.6 atoms, unless some polysulfide solvent which does not dissolve sulfur appreciably, e.g., Cellosolve, were added.

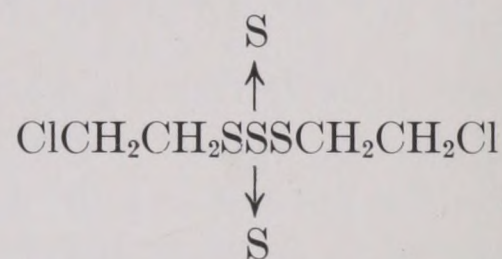
The pentasulfide exhibits relatively high stability compared with higher polysulfides and was obtained in a state of high purity after methods had been worked out to remove various impurities always present in the nonhydrolyzable Levinstein residues. Attempts to distill HS_5 or HS_5 -mustard mixtures by ordinary vacuum distillation result in degradation of HS_5 to HS_3 and HS_2 , both of which are readily distillable.

When HS_5 is subjected to steam-distillation, HS_3 is obtained in the distillate, and the nondistillable residue consists of polysulfides higher than HS_5 . Autosulfurization of HS_5 appears to take place under these conditions, the removal of the volatile HS_3 forcing the reaction to proceed. Higher polysulfides prepared in this way are similar to the polysulfides isolated from fresh Levinstein mustard in that they slowly deposit sulfur over a period of weeks. This deposition of sulfur can be accelerated by the usual stripping methods to reproduce HS_5 . By steam-distilling the impure polysulfide from Levinstein mustard for a short time to remove volatile impurities such as the disulfide and subsequently stripping the higher polysulfide residues with Cellosolve, pure HS_5 can be obtained as a light amber, slightly viscous liquid with a much less pronounced odor than that of the unpurified material. The molecular formula, $\text{C}_4\text{H}_8\text{Cl}_2\text{S}_5$, has been verified by ultimate analysis.

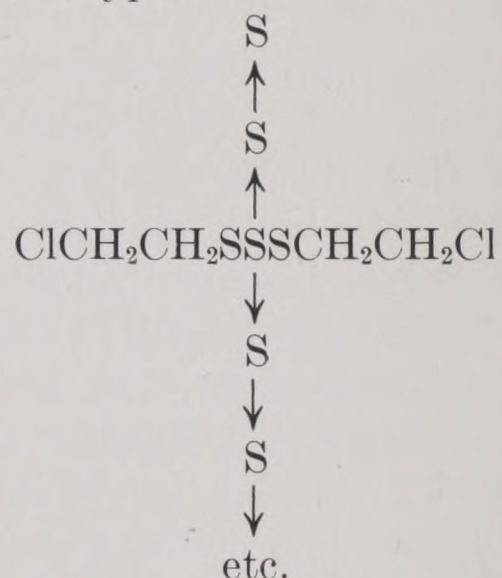
Although it is stable to moist ammonia in the ab-

sence of a solvent, HS_5 is stripped to HS_3 by moist ammonia in the presence of a solvent such as ether or Cellosolve. Metallic mercury also strips sulfur from HS_5 , producing HS_3 and HS_2 , although the rate at which the stripping proceeds is much less in going from HS_3 to HS_2 than in converting HS_5 to HS_3 .³⁵

The stability of HS_5 and HS_3 and the degradation of HS_5 to yield HS_3 indicate that these compounds may be structurally related. HS_2 is known to be a linear disulfide. One sulfur atom may be added with difficulty to HS_2 but following this two additional atoms enter with comparative ease to give HS_5 . Degradation of this HS_5 gives HS_3 . These phenomena can be accounted for most easily by assuming that a sulfur atom enters the HS_2 molecule to produce a linear trisulfide of the structure $\text{ClCH}_2\text{CH}_2\text{SSSCH}_2\text{CH}_2\text{Cl}$ and that this molecule is then sulfurized to produce HS_5 of the structure



The higher polysulfides could be accounted for by structures of the type



which would be in accord with the natural chain-forming tendency of sulfur atoms, and the limited stability of the higher polysulfides.³⁵

During a study of the composition of British Levinstein H, British investigators arrived at similar conclusions.¹⁹¹ When treated with cold acetone their sample, prepared at Sutton Oak, produced a milky suspension which gradually deposited crystalline sulfur. Only a small amount of sulfur was produced, apparently that in solution in the Levinstein mustard. Attempts to distill the higher boiling fractions of Levinstein failed because of the decomposition of a "labile polysulfide" which could be removed by

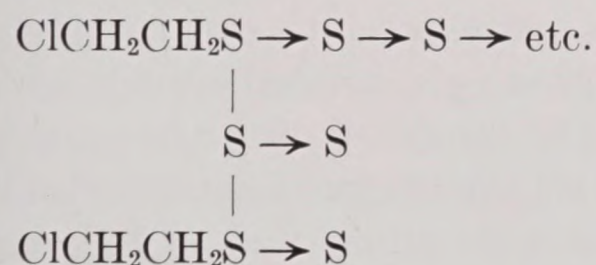
preliminary refluxing of the higher boiling fractions with acetone. After this period of refluxing, the precipitated sulfur was removed by filtration. The resulting material was found to be distillable under reduced pressure without decomposition to give HS_2 and HS_3 , which were compared with synthetic samples.

Examination of the labile polysulfide obtained as a residue from the preliminary low-temperature distillation of Levinstein mustard revealed it to be a yellow, viscous oil, nearly insoluble in alcohol, petroleum ether, and acetone, but soluble in benzene, chloroform, and carbon disulfide. Similar material could be obtained as a residue by extracting Levinstein mustard with methanol. The polysulfide could be broken down partially to "2,2'-dichlorodiethyl trisulfide and sulfur . . . by heating at about 100 C for a few hours. . . ." This change could also be brought about slowly but completely by heating the labile polysulfide under reflux in the presence of acetone, since one of the products of this breakdown is soluble in acetone and the other, sulfur, is not.

Synthesis of the labile polysulfide was accomplished by heating the trisulfide with 3 gram-atoms of sulfur at 110 C for several hours. After extraction of the product with methanol to remove unchanged trisulfide and separation by filtration from a small amount of unreacted sulfur, an oil resembling the original labile polysulfide remained.

The trisulfide could be obtained by distillation of the methyl alcohol extracts of Levinstein mustard without the formation of much sulfur, indicating that some trisulfide exists uncombined in the original mixture.

The British investigators suggested that the labile polysulfide probably contains compounds of the type



or perhaps, since mono- and disulfides do not form polysulfides when treated in the same manner, structures with the additional sulfur atoms branching from the central S of HS_3 .

HS_4 also appears to be a true level of stability, since HS_5 from different sources is stripped to this level on refluxing in acetone, and HS_3 on heating with sulfur in boiling acetone for several days undergoes

sulfurization to a product whose composition approaches that of HS_4 .³⁵

A number of less direct lines of evidence point to higher polysulfides as the principal impurities in Levinstein H.

Thus, in a series of studies on fractional melting of Levinstein H samples, the densities and refractive indices of a number of fractions as functions of H content were recorded.²¹ If the densities and refractive indices of various Levinstein H fractions are plotted against their H contents, straight lines are obtained, which can be extrapolated to give the corresponding values for the impurity treated as a single component. From the equations of these two lines it is possible to eliminate the H content, giving an expression relating density and refractive index. Experimentally determined values for the refractive indices and densities of synthetic samples of HS_2 , HS_3 , and HS_5 all fell on or very close to this curve, but not so far out as the extrapolated values for the impurity, which were also on the curve. The indications were that the impurity was a *bis*(β -chloroethyl) polysulfide containing six or seven sulfur atoms.

Two independent investigations upon the composition of solvent extracts and steam distillates of Levinstein H using cryoscopic methods have been reported.^{85,257} In each, measurements of melting point and of average molecular weight, determined cryoscopically, of the samples have allowed calculation of the mole fraction of the impurity and thus its average molecular weight. In one investigation²⁵⁷ the average molecular weights of the impurities in several extracts were 311, 291, and 250, corresponding roughly to HS_6 , HS_5 , and HS_4 . In the other,⁸⁵ the impurities in crude Levinstein were found to have an average molecular weight corresponding approximately to HS_7 , whereas the average molecular weight of the impurities in Levinstein stripped with moist ammonia or completely extracted with pentane corresponded approximately to HS_5 . The results with steam-distilled material are divergent, indicating in one case²⁵⁷ that low molecular weight impurities are present in sufficient quantities to bring the average molecular weight of the mixture below that of pure H, whereas in the other case⁸⁵ significant amounts of HS_2 are indicated.

Molecular distillations carried out on three samples of Levinstein H at temperatures not exceeding 30 C also indicate that the residues remaining after such distillations, during which the sample is not likely to have altered, are composed of polysulfides of com-

TABLE 1.³⁵ Mixtures of H and HS_x.

Mixtures of H and HS _x which have the composition of the mixture one molecule of H plus one atom of sulfur						Mixtures of H and HS ₅ produced by stripping			
Mixture*	Mole ratio	Mole (per cent)	Weight (per cent)	Calcd. fp† in C	Weight (per cent) strippable sulfur	Mixture*	Weight (per cent)	Chlorine (per cent)	Sulfur (per cent)
1. H HS ₅	3 1	75 25	62.5 37.5	5.6	0	H HS ₅	62.5 37.5	37.2	33.5
2. H HS ₆	4 1	80 20	66.8 33.2	7.2	3.4	H HS ₅	69.0 31.0	38.5	31.2
3. H HS ₇	5 1	83.3 16.7	69.5 30.5	8.5	5.5	H HS ₅	73.6 26.4	39.4	29.6
4. H HS ₈	6 1	85.7 14.3	71.5 28.5	9.1	7.2	H HS ₅	76.9 23.1	40.0	28.3
5. H HS ₉	7 1	87.5 12.5	72.9 27.1	9.7	8.4	H HS ₅	79.6 20.4	40.6	27.4
6. H HS ₁₀	8 1	88.9 11.1	74.1 25.9	10.4	9.3	H HS ₅	81.7 18.3	41.1	26.7

* All these mixtures of H + HS_x contain 33.5 per cent S and 37.1 per cent Cl.

† These freezing points were interpolated from the "Mean Curve."¹³

positions and molecular weights corresponding to HS₅, HS₆, and HS₇. Indices of refraction of the residues from two of these fractions appear to be in agreement with this assumption.⁵⁷

In summary there appears to be much trustworthy evidence indicating that Levinstein mustard is chiefly a solution of *bis*(β-chloroethyl) sulfide and polysulfides of varying composition and stability and of the general formula, (ClCH₂CH₂)₂S_x. Part of the excess sulfur is used in forming the stable linear trisulfide skeleton and this sulfur cannot be removed by ordinary methods of stripping. The pentasulfide represents another stable level. Aged Levinstein mustard or Levinstein mustard stripped by the usual methods involving moist ammonia, consists essentially of a mixture of *bis*(β-chloroethyl) sulfide and HS₅. Consideration of the amount of sulfur formed by the stripping process, the amount of pentasulfide which can be isolated from Levinstein, and the freezing point of fresh Levinstein leads to the conclusion that the polysulfide in freshly prepared Levinstein mustard may have an average composition varying from that of the hexasulfide to that of the decasulfide. Usually the polysulfide has a composition corresponding approximately to a heptasulfide. The composition of the polysulfide as well as its concentration in fresh Levinstein mustard depends upon the conditions under which the reaction was carried out, especially upon the temperature and upon the rate

of agitation, amount of seed charge, and rate of addition of ethylene. Very likely many other factors, such as previous history of the sulfur monochloride, also have important effects.

If there is no sulfur precipitated during the reaction, there must be a definite relationship between the composition of the polysulfide and its concentration, since one sulfur atom is produced each time a molecule of *bis*(β-chloroethyl) sulfide is formed and this sulfur must nearly all be present as polysulfides. Thus the higher the sulfur content of the polysulfide, the lower need be its mole concentration. Table 1 illustrates this relationship. The sulfur and chlorine contents of all the mixtures before stripping are 33.5 per cent and 37.1 per cent, respectively, as required to correspond to one atom of sulfur per molecule of *bis*(β-chloroethyl) sulfide with one atom of sulfur. The table lists other properties, based on theory, which the original mixtures should have. By making the assumption that H-HS₅ mixtures are produced by stripping it is possible to tabulate the final content by weight of each constituent and the sulfur and chlorine content of the resulting mixtures.

The theoretical values which appear in the table are in excellent agreement with the vast quantity of experimental data on Levinstein mustard compiled at Edgewood Arsenal and other Chemical Warfare Service laboratories. Many of these data are summarized elsewhere.^{35,111}

bis(β -Chloroethyl) disulfide was formerly thought to be present in Levinstein H in amounts as high as 6 per cent¹⁰⁰ but methods which do not result in the degradation of higher polysulfides indicate a much smaller percentage for this component.^{35,62} Analysis of the stripped nonhydrolyzable residue from Levinstein H (composed of HS_2 and HS_5) and of the steam distillate from this (composed of HS_2 and HS_3) by comparison of their refractive indices with curves prepared from known mixtures indicate 1 to 1.8 per cent of HS_2 in crude Levinstein H,³⁵ which is in good agreement with a value obtained by analysis of a fraction obtained by low-temperature molecular distillation of Levinstein H.⁶²

In addition to the higher polysulfides, several other impurities are usually present in significant amounts in Levinstein H. Of these, one of the most important is 1,2,2'-trichlorodiethyl sulfide ("trichloro H"). This material is formed by the chlorinating action of sulfur chlorides, particularly in processes where higher temperatures are encountered. The pressure instability of both Levinstein H and British sulfur dichloride mustards has been ascribed to the ready loss of hydrogen chloride from this and similar substances.^{21,235} The product of dehydrohalogenation, β -chloroethyl β' -chlorovinyl sulfide (CECVS), is thus also an impurity.

Evidence for the presence of trichloro H in crude Levinstein H is mostly indirect but can be summarized as follows:²¹

1. Levinstein H contains an acidic impurity or an acid-forming impurity which is usually expressed as HCl, but which cannot be removed by air blowing as can HCl.

2. Vacuum distillation of Levinstein H is accompanied by a weight loss, at least a part of which is HCl. The main impurity in the distillate appears to be β -chloroethyl β' -chlorovinyl sulfide.

3. Experiments on fractional melting of crude Levinstein H indicate that an impurity appearing in the first (lowest melting) fractions also appears in the distillate of such fractions. This does not indicate what the impurity is, but the inference is that β -chloroethyl β' -chlorovinyl sulfide is present in the distillates, whereas its precursor, trichloro H, is present in the original material. Semiquantitative estimates indicate that it may make up as much as 6 per cent of the crude H.²¹

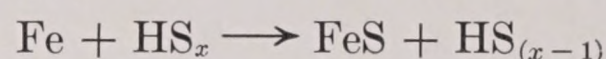
Both trichloro H and β -chloroethyl β' -chlorovinyl sulfide have been synthesized, the latter by an unambiguous method making use of the addition of

β -chloroethylsulfenyl chloride to acetylene as well as by dehydrochlorination of trichloro H.³⁵

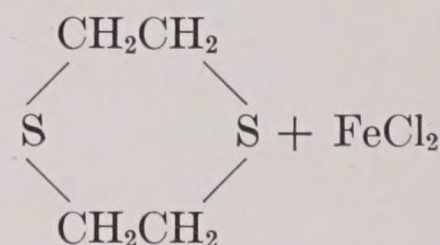
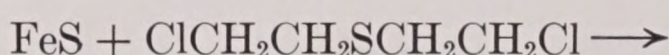
In addition to the above impurities, Levinstein H contains varying small amounts of a number of other substances. Among these may be mentioned small amounts of volatile materials, including methane,⁵⁷ hydrogen chloride,⁵⁷ diethyl ether,⁵⁷ chlorinated hydrocarbons, possibly 2-chlorobutane or ethylene chloride,^{21,35} and sulfur. Levinstein H which has been prepared or stored in iron always contains dissolved iron in greatly varying amounts, depending upon the length and conditions of storage and the conditions of the original reaction. One of the actions of hexamine as a stabilizer is to remove this iron.³²

The storage of Levinstein mustard in the presence of iron at 65 C leads to the formation of an iron-containing polymer. This polymer has been shown to be a sulfonium salt of *bis*(β -chloroethyl) sulfide, dithiane, and ferrous chloride,¹⁰ arising by the following mechanism:

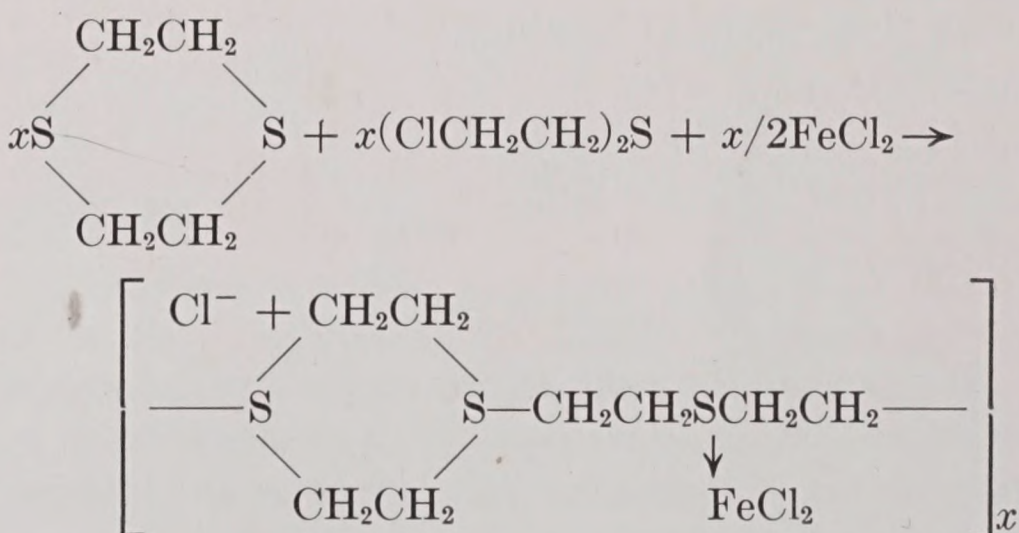
Ferrous sulfide is formed by the action of polysulfide on iron,



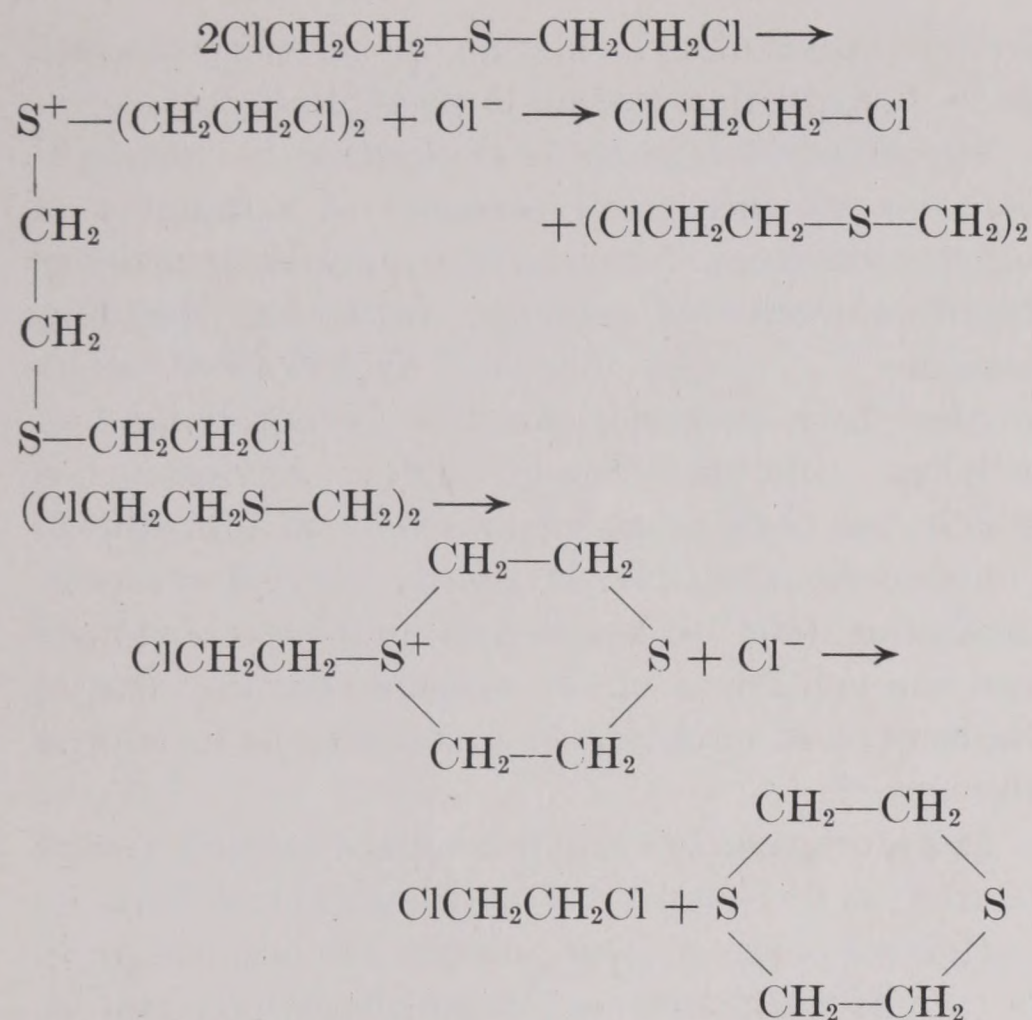
and the ferrous sulfide transforms mustard into dithiane.



This combines with mustard and the ferrous chloride to form the polymer³⁵



Levinstein H which has been heated or which has undergone storage at elevated temperatures also contains small amounts of Q, dithiane, and ethylene chloride, formed by the following series of reaction:³¹



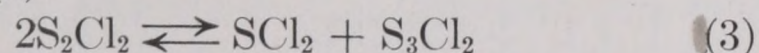
The presence of Q in stored Levinstein was independently indicated by a series of toxicological observations on the increased vesicancy of samples of H stored at 65 C and above. ^{60h,i,l}

5.2.5 The Mechanism of Formation of Levinstein H

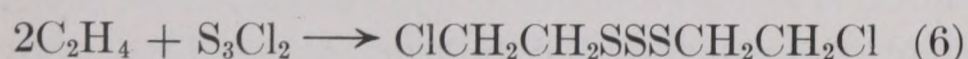
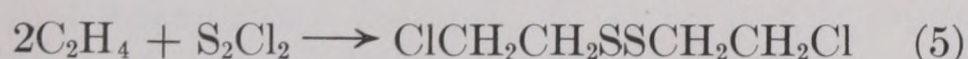
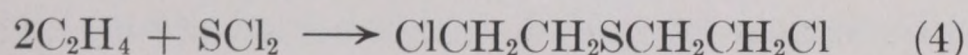
Any mechanism which is proposed to account for the formation of Levinstein mustard must explain ³⁵

1. The formation of *bis*(β -chloroethyl) sulfide.
 2. The formation of polysulfides derived from the linear HS₃ skeleton.
 3. The absence of more than traces of the disulfide.
 4. The absence of more than traces of free sulfur.
- A mechanism which fulfills these conditions has been developed.

There is chemical and physical evidence ³⁵ to support the assumption that sulfur monochloride disproportionates, to a slight extent at least, to give sulfur dichloride and sulfur tritradichloride according to equation (3).

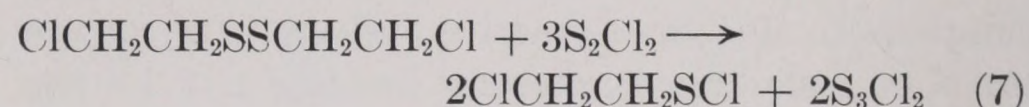


It is beyond the scope of this review to discuss this evidence, but, if this equilibrium exists, then it is possible for all three materials to react with ethylene according to equations (4), (5), and (6).

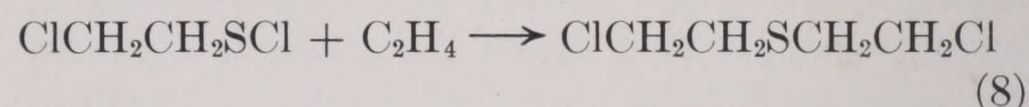


Equation (6) would apply equally well if higher polythio sulfur chlorides such as S₄Cl₂, etc., were formed.

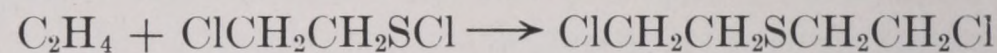
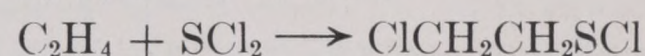
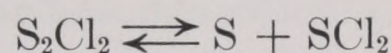
The disulfide (HS₂) produced according to equation (5) has been shown to react readily with sulfur monochloride to produce β -chloroethylsulfenyl chloride and sulfur tritradichloride according to equation (7). ³⁵



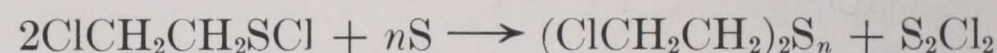
These products can react with ethylene rapidly to yield *bis*(β -chloroethyl) sulfide and HS₃, or, in the reaction used to demonstrate the presence of the sulfenyl chloride, with cyclohexene to yield β -chloroethyl β' -chlorocyclohexyl sulfide.



The series of reactions corresponding to equations (5), (7), and (8) are reminiscent of the mechanism originally proposed by Conant ²⁶⁴ in 1920. According to Conant, the reaction passed through the following phases:



These reactions were accompanied by a secondary reaction

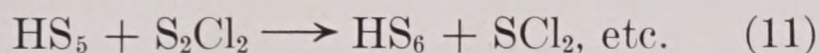
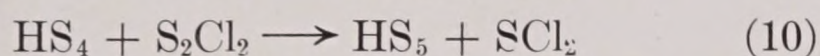
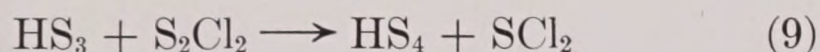


Convincing evidence of either the existence of β -chloroethylsulfenyl chloride or its participation in the reaction was not advanced until 1943 ⁴⁹ when the pure material was prepared by chlorinolysis of HS₂ and HS₃. It reacts very readily with ethylene and with cyclohexene to produce H and β -chloroethyl β' -chlorocyclohexyl sulfide, respectively. β -Chloroethylsulfenyl chloride also appears to be formed by the action of sulfur monochloride on HS₂ and by the action of sulfur dichloride on HS₂ and HS₃, since β -chloroethyl β' -chlorocyclohexyl sulfide can be obtained by the addition of cyclohexene to these reaction mixtures. ³⁵

As additional evidence that β -chloroethylsulfenyl chloride is an intermediate in the Levinstein reaction, it has been shown ³⁵ that an equimolecular mixture of ethylene and cyclohexene vapor used in the Levinstein process instead of pure ethylene produces, in addition to *bis*(β -chloroethyl) sulfide and *bis*-2-chlorocyclohexyl sulfide, the mixed sulfide, β -chloro-

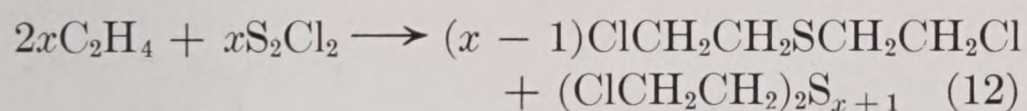
ethyl 2-chlorocyclohexyl sulfide in the expected mole ratio of approximately 1/1/2 respectively. Likewise, an equimolar mixture of ethylene and cyclohexene vapor passed through a solution of sulfur dichloride in mustard yields the same three products, indicating the two-step nature of the reaction of ethylene with sulfur dichloride.

The HS_3 formed according to equation (6) has been shown to be sulfurized readily at 30–35 C by sulfur monochloride to higher polysulfides [equations (9), (10), and (11)] and again sulfur dichloride is the other product of the reaction. It immediately reacts with ethylene to produce more *bis*- β -chloroethyl sulfide.



Because sulfur dichloride is introduced into the reaction mixture as a product of other reactions or as a result of the initial equilibrium, and since it reacts rapidly with ethylene, its concentration is low as long as excess ethylene is present. For this reason, the problem of overchlorination with resulting pressure instability of the product is not so significant in the Levinstein process as in the processes involving the use of sulfur dichloride directly.

The net result of this series of reactions is to produce a mixture of *bis*- β -chloroethyl sulfide and polysulfides corresponding to the monosulfide. The overall equation for the Levinstein reaction, (12), is thus:



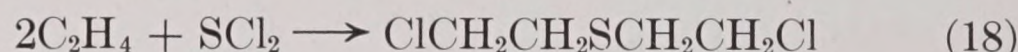
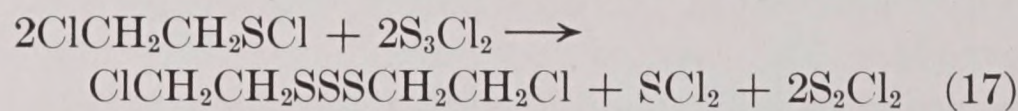
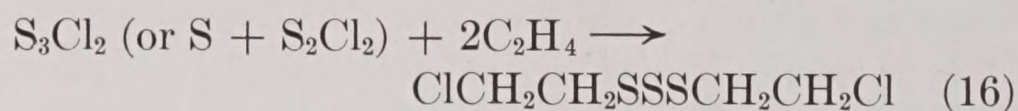
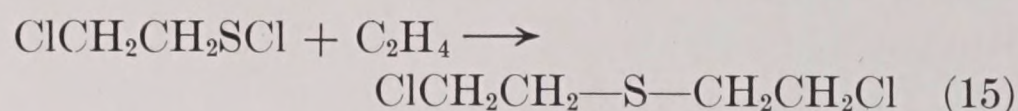
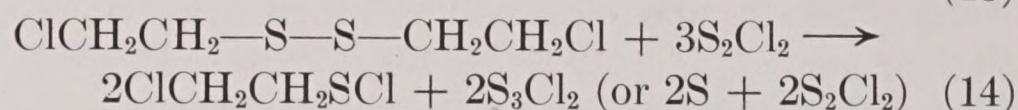
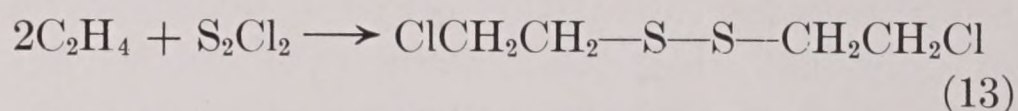
The amount of impurity (as polysulfides) in the resulting Levinstein mustard depends upon the rate of formation of HS_3 and on the rate of sulfurization of HS_3 by sulfur monochloride.³⁵

If it is assumed that the effect of temperature on this series of reactions is to increase the rate of all the reactions but that this is partially offset by the decreased solubility of ethylene at higher temperatures, then the net effect is to increase the rate of the sulfurization reactions relative to those involving ethylene. As fast as HS_3 molecules are formed, they are sulfurized to higher polysulfides, and, since the addition of each sulfur atom results indirectly in the formation of one molecule of H (through SCl_2), the product formed at higher temperatures should be high in H content and in very high unstable poly-

sulfides which readily lose sulfur. At low temperatures sulfurization is slower, more HS_3 is formed, it is sulfurized to a lower degree, and less H results. This interpretation is in excellent agreement with the known characteristics of high-temperature (60 C process) and low-temperature (Levinstein process) H.

The reaction of sulfur monochloride with propylene to give "propyl mustard," long believed²¹⁶ and now known⁴¹ to have the "normal" $\text{CH}_3\text{CH}(\text{Cl})\text{CH}_2-\text{S}-\text{CH}_2\text{CH}(\text{Cl})\text{CH}_3$ rather than the "iso" structure, can also be accommodated to the above reaction scheme, since it has been shown that β -chloroethylsulfenyl chloride, and by inference other sulfenyl chlorides, add to propylene in accordance with Markownikoff's rule to give the "normal" structure.⁴¹

It should be noted that even if the initial assumption of the scheme, i.e., the disproportionation of sulfur monochloride to sulfur dichloride and sulfur tritradichloride is not valid, the following series of reactions provide a satisfactory mechanism:



Sulfurization of HS_3 to higher polysulfides by sulfur monochloride with the simultaneous production of H would proceed as already described.³⁵

The assumption that sulfur dichloride can react in both linear and angular forms to give linear disulfides and thiosulfoxides is not considered likely, since it has not been possible to isolate a thiosulfoxide or to obtain convincing evidence of the existence of this type of compound. The possibility cannot be excluded, however, since in one case it has been possible to isolate material of composition corresponding to a monosulfide plus varying amounts of sulfur which appeared to be present in a very labile form.^{35,47} Evidence that trisulfides also exist in the linear form has been summarized.⁴⁷

5.2.6 The Purification and Stabilization of Levinstein H

The storage and corrosion stability of Levinstein H as ordinarily produced is not entirely satisfactory and it cannot be thickened for use as a spray. A review of the immense amount of effort which has been devoted to the study of its stabilization is beyond the scope of this report, and it is possible here only to describe the present position.

A number of methods have been examined for the purification of Levinstein H, including treatment with ammonia, heat treatment, treatment with silica gel or charcoal, distillation under various pressures, flash distillation, carrier distillation using both steam and organic liquids, solvent extraction,¹¹¹ and crystal fractionation.²² Of these, only vacuum distillation, steam distillation, and solvent extraction appear feasible for use on a plant scale. Pilot plant studies of all three have been carried out.^{25,86,160,254} Without a detailed discussion of the advantages and disadvantages of each method, it can be said that solvent extraction using commercial pentane gives excellent recovery (up to 95 per cent of the available H) of a product which contains 92.5 per cent H but whose pressure and corrosion stability is inferior to that of steam- or vacuum-distilled material; superheated steam distillation which requires acid-proof equipment produces more stable material of 95 per cent or better purity in recoveries of 80 per cent; whereas vacuum distillation of water-washed Levinstein H produces material of exceptionally good stability and 95–96 per cent purity with 86 per cent recovery. The vacuum distillation has the advantage of using existing plant facilities and appears to be the method of choice. Washing with water removes iron salts and acid and is an essential step; vacuum distillation of unwashed Levinstein H is unsatisfactory as a purification measure.

A detailed description of the efforts to improve the pressure and corrosion stability of Levinstein H and to retard its decomposition without resorting to extensive purification procedures cannot be given here. As a result of these efforts, which have included studies on a variety of stabilizers, the addition of 1 per cent of hexamethylenetetramine (hexamine), whose efficacy as a stabilizer for Levinstein H was first recognized by the CWS-MIT Development Laboratory,⁸⁴ to all munitions charged with Levinstein H and to all storage containers is now standard. This treatment confers satisfactory storage stability, as regards decomposition, corrosion, and pressure de-

velopment, on Levinstein H, but is not sufficient to render this mustard satisfactory for thickening with polymethylmethacrylate. For this purpose it is necessary to use Levinstein H whose iron content is below 1 per cent, to add 1 per cent hexamine and 2 per cent nitrogen bases (either coal tar or petroleum), and to store the thickened material in lacquered containers.^{24,32,87,89,90,114,143,152}

The stability of Levinstein mustard which has been purified by various methods is also improved by the addition of 1 per cent hexamine.

The precipitate formed when hexamine is added to Levinstein H contains hydrogen chloride, iron chlorides, and sulfur. The action of hexamine as a stabilizer appears to be due to its ability to reduce iron content and acid, and has been ascribed to these factors and to its high stability and low basicity, which was believed to be responsible for its lack of compound formation with H.³² There is, however, disagreement as to the solubility of hexamine in H and as to the extent of compound formation between hexamine and H,^{32,152} and the mechanism of stabilization of Levinstein H by hexamine cannot be considered completely clear.

No attempt will be made here to describe the extensive work on the thickening of H for use as an airplane spray or in airburst munitions. This subject is treated in detail elsewhere.^c

5.2.7 Photosynthetic Methods for the Preparation of H and Analogs

As a result of work initiated in 1942 under Division 9 of NDRC, a novel method for the synthesis of H and particularly of more complex relatives of H, such as Q, has been developed. The method gives excellent yields, is capable of great flexibility, and has been applied successfully in a number of cases (see Table 2).^{59,277} The synthesis consists of the photochemical addition of hydrogen sulfide or mercaptans to vinyl chloride and related olefins in the presence of photoactivators.

The addition of hydrogen sulfide to vinyl chloride brought about by irradiation with ultraviolet light in the presence of peroxide catalysts was accomplished in 1942 by NDRC groups⁵⁹ but a publication appearing about this time indicates that the process had been studied earlier by a private group.²⁷⁷ Yields of 75–80 per cent accompanied by some β -chloroethylmercaptan and polymeric material can be ob-

^c See NDRC Division 11 Summary Technical Report.

TABLE 2. Compounds related to H prepared by photo-synthetic methods.

Compound	Reference
1. β -Chloroethyl ethyl sulfide	59
2. <i>bis</i> (β -Chloroethyl) sulfide (H)	59
3. β -Chloroethyl β' , β' -dichloroethyl sulfide (2-chloro H)	42
4. β -Chloroethyl β' -hydroxyethyl sulfide (CH)	43, 142
5. β -(β -Chloroethylthio)ethylmercaptan	125
6. 1,2- <i>bis</i> (β -Fluoroethylthio)ethane	59
7. 1,2- <i>bis</i> (β -Chloroethylthio)ethane (Q)	59, 122 et seq.
8. 1,2- <i>bis</i> (β -Bromoethylthio)ethane	59
9. 1,3- <i>bis</i> (β -Chloroethyl)-2-chloropropane	59
10. 1,2- <i>bis</i> (β -Chloroethyl)-3-hydroxypropane	59
11. 2,3- <i>bis</i> (β -Chloroethyl)butane	59
12. <i>bis</i> (β -Chloroethylthioethyl) ether (T)	59, 131
13. <i>bis</i> (β -(β -Chloroethylthio)ethyl) sulfide (di H)	129
14. α , α' - <i>bis</i> (β -Chloroethylthio)- <i>p</i> -xylene	59
15. β -Fluoroethyl thiolacetate	59

tained. Illumination with ultraviolet light in the region 2,800–3,200 Å is effective; peroxides and benzoin, a known photoactivator in the ultraviolet, promote the reaction. Neither thermal nor catalytic activation will induce addition.⁵⁹

It has been shown²⁷⁷ that the photochemical addition of hydrogen sulfide to olefins proceeds according to Markownikoff's rule. Ultraviolet light alone was found to be sufficient for the reaction to take place, but photosensitizers extended the range of effective frequencies. In the case of the synthesis of H from hydrogen sulfide and vinyl chloride, both irradiation with ultraviolet light and the presence of a catalyst appear to be necessary.⁵⁹

The feasibility of synthesizing pure Q in high yields by the photochemical addition of ethanedithiol to vinyl chloride has been demonstrated⁵⁹ and the reaction has been exhaustively studied at Edgewood Arsenal.

The reaction is carried out as a batch process, and on a laboratory scale can be done either under atmospheric pressure at the boiling point of vinyl chloride (–13.6°C) or at room temperature under approximately 4 atmospheres. Yields are 90 per cent in the first case and quantitative in the second when the irradiation is supplied by an S-4 General Electric mercury vapor lamp and either benzoyl peroxide or sodium percarbonate is present. The reaction can also be carried out at room temperature by bubbling vinyl chloride through the reaction mixture.¹²² Results at 75°C have been definitely inferior to those obtained at lower temperatures.¹⁴⁷ The presence of a solvent such as benzene is advantageous since vinyl chloride is not very soluble in ethanedithiol.^{125,147}

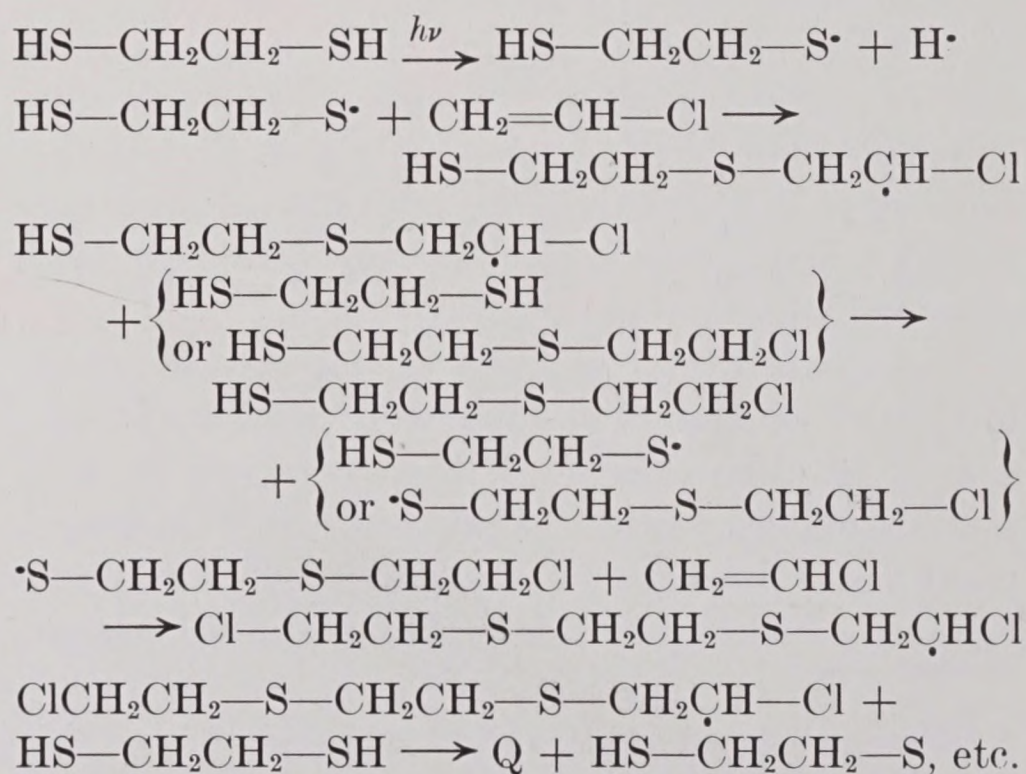
Methanol can also be used but the product separates from this solvent as a second phase containing both methanol and ethanedithiol, and must be stripped. The possibility of using methanol in a continuous system has been recognized,¹⁴⁷ but no development work along this line has been reported.

The Q produced is of excellent quality and melts at 53.6°C,¹²² but has a pronounced odor of ethanedithiol, which may be removed by treatment with arsenic trichloride or L.¹³⁵

The heat of the photochemical reaction, which is exothermic, has not been measured but calculations from bond energies indicate a value of about 18 kcal/mole.¹²²

In contrast to the addition of hydrogen sulfide to vinyl chloride, the addition of ethanedithiol proceeds without promoters or photosensitizers.⁵⁹ However, the presence of these is very desirable and a number have been examined, particularly with respect to the reduction of the light requirements of the reaction.¹⁴⁷ The most effective are peroxides of various types and disulfides.^{59,135,147} Diphenyl disulfide is far superior to any of 18 other disulfide catalysts tried when the reaction is carried out in sealed tubes, but is no better than diamyl disulfide when used at atmospheric pressure.¹⁴⁷ Oxygen is ineffective.¹³⁸

The reaction appears to proceed by a chain mechanism initiated, in the uncatalyzed case, by the photodissociation of ethanedithiol into radicals which attack the vinyl chloride molecule. Quantum efficiencies of about 1,000 are observed.¹⁴⁷ Chain propagation occurs through the alkyl sulfide radical:



In the case of catalysis by photosensitizers, the initial photodissociation is of the catalyst molecule, and the

radical formed initiates a radical chain similar to the one above. Thus, the problem of reducing the light requirements of the reaction is resolved into one of finding photosensitizers which will yield radicals at longer wavelengths than that required for the photodissociation of ethanedithiol.^d Disulfides absorb light even more strongly than ethanedithiol in the near ultraviolet, and provide alkyl sulfide radicals at longer wavelengths. Thus the 3,650 Å mercury line is effective if diamyl disulfide is used as a photosensitizer in the Q reaction.¹⁴⁷ The diaryl disulfides, which are known to be easily dissociable,²⁷⁵ absorb at even longer wavelengths (up to 4,200 Å for diphenyl disulfide) and are the most effective photosensitizers. Thus with diphenyl disulfide, which has been thoroughly investigated, excellent conversions are obtained with 300-watt Mazda lamps, and fairly good conversion is possible even with a 60-watt Mazda lamp. Among other easily dissociable compounds examined, the triaryl free radicals did not prove to be effective promoters.

A novel "ionic chain" mechanism for this reaction received serious early consideration by CWS workers¹³⁵ but was later abandoned.¹⁴⁷ An early view that vinyl chloride was activated by light¹²² was similarly abandoned, since vinyl chloride does not absorb in the ultraviolet.¹⁴⁷

Substances normally effective in acid-base catalyzed reactions such as boron trifluoride, phosphoric acid, hydrogen ion, triethanolamine, and water are ineffective as promoters in the photosynthesis of Q.^{135,147}

A number of materials, e.g., copper, iron, tin, carbon, sulfur, and polysulfides, exert an inhibiting effect on the reaction, in some cases enough to completely prevent reaction,^{59,135} and considerable difficulty has been experienced in scaling up the reaction because of this sensitivity to inhibition. Laboratory results indicate that stainless steel, aluminum, lead, zinc, and silver are not injurious.¹³⁵

The ethanedithiol required in the photosynthesis of Q can be prepared in 76 per cent yield by thionation of ethylene chloride with aqueous sodium sulfhydrate under hydrogen sulfide pressure.⁵⁸ On a semi-plant scale, the expensive hydrogen sulfide has been replaced by carbon dioxide, which produces hydrogen

sulfide from the sodium sulfhydrate.²⁹ The efficiency of this process for production of ethanedithiol on a small-scale manufacturing basis has been demonstrated by the production of about 1,600 lb. The conditions were not studied exhaustively, but a cheap, practical process easily adaptable to large-scale manufacture was developed.²⁹

5.3 TABULATION OF ORGANIC SULFUR COMPOUNDS EXAMINED AS CANDIDATE CHEMICAL WARFARE AGENTS

The many analogs of H which have been studied by investigators in the United States, Great Britain, and Canada are listed in Table 3, with references to the reports on their preparation and toxicological action. The closely related nitrogen mustard series is tabulated in Chapter 6. It can be stated that the study of analogs in the sulfur mustard series has not disclosed compounds superior to H, Q, and T in potential general usefulness as chemical warfare agents. In the study of the mechanism of action of agents in this category, however, as will be noted later, extensive use has been made of the data on the chemical and toxicological properties of the compounds related to H.

5.4 PROPERTIES OF H, Q, AND T

5.4.1 Physical Properties

The physical properties of H, Q, and T which have the most direct bearing on the effectiveness of these agents as war gases are the following:

	H	Q	T
Density (liquid) g/ml at 25 C	1.27		1.24 ²³¹
Boiling point C	217	353 (calc.) ⁷⁴	120/0.02 mm ²³¹
Freezing point C	14	56-57 ¹⁸	10 ²³¹
Volatility mg/l 25 C	0.96 ^{60e}	0.0004 ⁹⁶	0.0028 ^{60m}

Whereas H has sufficient volatility to yield injurious vapor dosages, neither Q nor T presents vapor hazards. The vesicant action of Q and T depends upon contact between the skin and the liquid or particulate agent. The low volatility of these two compounds gives them a much longer persistence than H as potential hazards on contaminated terrain.¹⁷³

^d The uncatalyzed addition of ethanedithiol to vinyl chloride occurs at wavelengths of 3,125 Å and at all wavelengths below this, in fair agreement with a probable threshold wavelength (calculated from bond strengths) of 3,254 Å for the photodissociation of ethanedithiol.¹⁴⁷

TABLE 3. Organic sulfur compounds examined as candidate chemical warfare agents.†

The general arrangement of the table is as follows: (1) mercaptans and derivatives; (2) sulfides and sulfide ethers; (3) polysulfides; (4) sulfoxides; (5) sulfones; (6) derivatives of thioacids; (7) sulfonium salts; (8) derivatives of sulfinic and sulfonic acids; (9) sulfur compounds of unknown constitution.

The following abbreviations are used: n_D^t , refractive index at t C; $d_{t_2}^{t_1}$, specific gravity at t_1 C in reference to water at t_2 C; mp, melting point in C; bp^p, boiling point in C at p mm Hg; vp^t, vapor pressure in mm Hg at t C; and vol^t, saturation concentration (volatility) in mg/l at t C.

Centigrade scale is used throughout the table.

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
1. β -Fluoroethylmercaptan	59	bp ²²⁵	38.5°	59	34
	...	n_D^{25}	1.4290	59	...
2. β -Chloroethylmercaptan	16, 53	bp ⁷⁶⁰	114–120°	16	34, 54
3. β -Hydroxyethylmercaptan	16	bp ⁹	47–50°	16	...
4. 1,3-Dichloro-2-mercaptopropane	61d			...	34, 54
5. 3-Mercapto-1,2-propylene sulfide	14			...	34
6. Ethanedithiol	29, 58	bp ⁵⁰	65–68°	58	34
	...	n_D^{25}	1.5570	58	...
	...	d_4^{25}	1.1182	58	...
7. 1-Chloro-2,3-dimercaptopropane	...			...	34, 54
8. 1-Hydroxy-2,3-dimercaptopropane* (DTH) (BAL)	14, 15, 237	bp ¹⁰	115° ± 1	14	242b
	...	n_D^{25}	1.570–1.573	14	...
	...	d_4^{25}	1.238–1.240	14	...
9. 2,3-Dimercaptopropionic acid	...			...	34
10. bis(β -Mercaptoethyl) sulfide	8	bp ³	104–106°	8	...
11. 1,6-Hexanedithiol	...			...	34, 54
12. <i>o</i> -Aminothiophenol*	...			...	...
13. 2-(ω -Chloroacetamino)-5-methyl-thiophenol*	...			...	...
14. Trichloromethyl sulfenyl chloride* (perchloromethyl mercaptan)	271	bp ⁷⁶⁰	148–149°	278	247
	...	bp ⁵⁰	73°	278	...
	...	d^0	1.722	278	...
	...	vol ²⁰	18	278	...
15. β -Chloroethylsulfenyl chloride	35	bp ¹⁵	47–47.5°	35	34, 54
16. <i>o</i> -Nitrobenzenesulfenyl-bis(β -chloroethyl)amine	39	mp	104–105°	39	34
17. bis(β -Chloroethylmercapto) chloramine	61c			...	34
18. Sulfilimine of chloramine-T and H	65a	mp	144.6°	278	34
19. <i>tetrakis</i> - β -Chloroethylmercaptosilicon	61h	bp ^{1.2}	98°	61h	34
	...	d_4^{20}	1.390	61h	...
20. Tributylthioethoxy tin	5	bp ^{1.5}	126°	5	...
	...	d^{26}	1.132	5	...
21. Triethyl β -chlorothioethoxy lead	61d			...	34
22. Thallous β -chloroethylmercaptide	61f	mp	>300°	61f	...
23. bis(α -Chloromethyl) sulfide*	...			...	...
24. Methyl β -chloroethyl sulfide	11, 23	bp ²³	52–53°	11	34, 54, 247
	...	vol ²⁰	32.7	38	...
25. β -Hydroxyethyl methyl sulfide	23	bp ¹²	63–66°	23	...
26. α -Chloromethyl- β' -chloroethyl sulfide	...	bp ¹⁸	105°	247	247
27. 3-Chloro-1,2-propylene sulfide*	8	bp ⁵⁻⁷	38–40°	8	34, 54
	...	n_D^{20}	1.5127	8	...
	...	d_{16}^{16}	1.250	8	...
28. 3-Thiocyano-1,2-propylene sulfide	53			...	34
29. Methyl 2,2'-dichloroisopropyl sulfide*	...			...	...
30. Methyl 2,2'-dihydroxyisopropyl sulfide*	...			...	...
31. Divinyl sulfide*	...			...	...
32. bis-Hexachlorodivinyl sulfide	...			...	...
33. β -Chloroethyl vinyl sulfide*	...			...	...
34. β -Chloroethyl- α -chlorovinyl sulfide*	...			...	189

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

† The table includes sulfur compounds in which sulfur is linked to carbon with the exception of thiocyanates, which are included in Table 1, Chapter 14. Sulfur compounds containing either arsenic or phosphorus are included in Table 1, Chapter 7, and Table 1, Chapter 9, and have not been repeated here.

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TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
35. β -Chloroethyl β' -chlorovinyl sulfide*	35	bp ^{0.75}	46°	35	34
	...	bp ^{0.15}	30°	35	...
	...	mp	-24°	35	...
	...	n_D^{20}	1.5480	35	...
36. Equimolar-mixture of triethylphosphorus and diethyl sulfide	61b			...	
37. Ethyl β -chloroethyl sulfide*	53, 59	bp ^{43.5}	73-76°	59	34, 54
	...	n_D^{25}	1.4858	59	...
	...	vol ²⁰	16.57	38	...
38. β -Thiocyanodiethyl sulfide*	...			...	
39. β -Chloroethyl β' -fluoroethyl sulfide	64a	bp ³⁰	91.5-92.5°	64a	34, 54
	...	mp	-44°	64a	...
	...	n_D^{20}	1.4872	64a	...
	...	d_{20}^{20}	1.228	64a	...
40. <i>bis</i> (α -Chloroethyl) sulfide*	8	bp ¹⁵	58.5-59.5°	8	...
41. α -Chloroethyl β -chloroethyl sulfide*	...			...	
42. Mustard gas	See text		See text	...	See text
43. β -Chloroethyl β',β' -dichloroethyl sulfide (2-chloro H)	42	bp ^{0.05}	68-69°	42	34
	...	n_D^{20}	1.5380	42	...
44. <i>bis</i> (β,β' -Dichloroethyl) sulfide*	...			...	
45. <i>bis</i> (α,β,β' -Trichloroethyl) sulfide*	...			...	
46. <i>bis</i> (β -Bromoethyl) sulfide	53	bp ^{0.1}	91-93°	53	34, 54, 247
	...	mp	31-33°	53	...
47. <i>bis</i> (β -Iodoethyl) sulfide	...	mp	68-70°	247	247
48. β -Chloroethyl β' -cyanoethyl sulfide*	...			...	
49. <i>bis</i> (β -Cyanoethyl) sulfide*	...			...	
50. β -Chloroethyl β' -hydroxyethyl sulfide* (CH)	8, 43, 142	bp ^{0.6}	100°	43	...
	...	n_D^{20}	1.5188	43	...
51. <i>bis</i> (β -Hydroxyethyl) sulfide	Commercial			...	34
52. <i>bis</i> (β -Chloroformylethyl) sulfide	64a			...	34
53. <i>bis</i> (β -Chloroacetoxyethyl) sulfide*	...			...	
54. <i>bis</i> (β -Trichloroacetoxyethyl) sulfide*	...			...	
55. <i>bis</i> (β -Bromoacetoxyethyl) sulfide*	...			...	
56. β -Hydroxyethyl β' -cyanoethyl sulfide	61e	bp ¹⁹	186-188°	61e	...
	...	n_D^{20}	1.5101	61e	...
	...	d_4^{20}	1.143	61e	...
57. β -Chloroethylthioacetyl chloride*	...			...	
58. Ethyl β -chloroethylthioacetate*	...			...	
59. Thiodiglycolic acid	276	mp	129°	276	34
60. Methyl thiodiglycolate	11	bp ²	102-104°	11	...
	...	n_D^{20}	1.4748	11	...
	...	d_{25}^{25}	1.230	11	...
61. Allyl β -chloroethyl sulfide*	53	bp ¹⁴	64.5-65°	53	34, 243c
	...	vol ²⁰	6.63	38	...
62. Ethyl β -chloropropyl sulfide*	...			...	
63. Ethyl β, γ -dichloropropyl sulfide*	...			...	
64. β -Chloroethylpropyl sulfide*	...			...	243c
65. β -Chloroethyl β' -chloropropyl sulfide*	41			...	54, 189
66. β -Chloroethyl β',γ' -dichloropropyl sulfide*	...			...	
67. <i>p</i> -Aminophenyl-(γ -chloropropylthio)ethylamine hydrochloride*	...			...	
68. Acetonyl β -chloroethyl sulfide	53	bp ^{0.75}	76-85°	53	...
	...	mp	145-146°	53	...
69. β -Chloroethylbutyl sulfide*	...			...	243c
70. β -Chloroethyl α' -methyl- β' -chloropropyl sulfide*	...			...	
71. <i>bis</i> (Chloroallyl) sulfide*	...			...	
72. <i>bis</i> (γ -Chloroallyl) sulfide*	...			...	

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

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TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
73. <i>bis</i> (β -Bromoallyl) sulfide*	...			...	...
74. <i>bis</i> (β -Chloropropyl) sulfide* (propyl mustard)	41	bp ^{0.04}	107–108°	41	34, 54
75. <i>bis</i> (γ -Chloropropyl) sulfide*	...	<i>n</i> _D ²⁰	1.4903–1.4917	...	...
76. <i>bis</i> (β , γ -Dichloropropyl) sulfide*	8	bp ⁷	111–112°	...	...
77. Propyl β -hydroxy- γ -fluoropropyl sulfide	64e	bp ²²	105–107°	64e	34
78. <i>bis</i> (<i>s</i> -Dichloroisopropyl) sulfide	...			...	34, 54
79. <i>bis</i> (α -Chloro- α -acetoxypisopropyl) sulfide*	...			...	...
80. <i>bis</i> (β -Chlorobutyl)-3-sulfide	Commercial			...	34, 54
81. β -Chloroethyl 2-chlorocyclopentyl sulfide	53	bp ^{0.15}	73°	53	34
	...	<i>n</i> _D ²⁰	1.5336	53	...
82. Ethyl 2,4,6-trichlorophenyl sulfide*	...			...	...
83. <i>p</i> -Ethylthiophenyldichlorostibine*	...			...	...
84. β -Chloroethylphenyl sulfide*	...			...	...
85. β -Chloroethyl <i>o</i> -nitrophenyl sulfide	53	mp	50°	53	34, 54
86. β -Chloroethyl <i>p</i> -nitrophenyl sulfide	53	mp	62°	53	34, 54
87. 1- β -Chloroethylthio-2,4-diaminobenzene hydrochloride*	...			...	...
88. <i>p</i> (β -Chloroethylthio)phenyldichlorostibine*	...			...	...
89. β -Chloroethylcyclohexyl sulfide	61f	bp ⁸⁰	161–163°	61f	34, 54
90. β -Chloroethyl β' -chlorocyclohexyl sulfide*	35, 53	bp ^{0.7}	108–109°	53	34
	...	<i>n</i> _D ²⁰	1.5382	53	...
	...	vol ²⁰	0.0474	38	...
91. Reaction product of butadiene and sulfur dichloride	...			...	...
92. <i>bis</i> (α -Methyl- β -chloropropyl) sulfide*	182	bp ⁵	89°	...	182
93. <i>bis</i> [α -(Chloroaceto)- β -chloroethyl] sulfide*	...			...	...
94. β -Chloroethyl heptyl sulfide*	...			...	243c
95. β -Chloroethyl <i>p</i> -tolyl sulfide	53	bp ²	115°	53	34
	...	<i>n</i> _D ²²	1.5728	53	...
96. β -Chloroethyl benzyl sulfide	53	bp ^{0.05}	95–97°	53	34, 243c
	...	vol ²⁰	0.115	38	...
97. β -Chloroethyl nonyl sulfide*	...			...	243c
98. Diphenyl sulfide*	...			...	...
99. <i>bis</i> (β -Chlorocyclohexyl) sulfide*	53	mp	73.5°	53	34
100. β -Chloroethyl undecyl sulfide*	...			...	243c
101. β -Chloroethyl chloromethoxymethyl sulfide*	...			...	...
102. β -Hydroxyethyl chloromethoxymethyl sulfide*	...			...	...
103. 1-Methoxy-2-(β -chloroethylthio)ethane*	...			...	243c
104. 1-Methylthio-2-(β -chloroethylthio)ethane*	...			...	243c
105. <i>bis</i> (β -Chloroethylthio) methane*	53	mp	31–32°	53	34, 54
106. 2-Methyl-4-chloromethyl-1,3-dithiocyclopentane*	...			...	...
107. 1-(β -Chloroethylthio)-2-ethoxyethane*	...			...	243c, 189
108. 1-(β -Chloroethoxy)-2-(β -chloroethylthio)ethane*	...	bp ¹	120°	179	179
109. 1-(β -Chloroethylthio)-2-ethylthioethane*	...			...	243c
110. 1,2- <i>bis</i> (β -Fluoroethylthio)ethane	59	bp ^{0.2}	85°	59	...
111. 1,2- <i>bis</i> (β -Chloroethylthio)ethane* (Q)	See text	mp	54°	59	See text
112. 1,2- <i>bis</i> (β -Bromoethylthio)ethane	53, 59	mp	78–79°	53	34, 54
113. 1,2- <i>bis</i> (β -Cyanoethylthio)ethane*	...			...	...
114. 1,2- <i>bis</i> (β -Hydroxyethylthio)ethane*	...			...	...
115. 1,2- <i>bis</i> (β -Chloroethylthio) acetaldehyde*	...			...	...
116. 2,2-Dimethyl-4-chloromethyl-1,3-dithiocyclopentane*	...			...	243c
117. 1-(β -Chloroethylthio)-2-propoxyethane*	...			...	243c
118. 1-(β -Chloroethylthio)-2-propylthioethane*	...			...	243c
119. 1,2- <i>bis</i> (β -Chloroethylthio)propane	8	bp ^{0.5}	139–142°	8	34, 54
	...	<i>d</i> ₄ ²⁵	1.233	8	...
120. 2,3- <i>bis</i> (β -Chloroethylmercapto)-1-chloropropane	59			...	34
121. 1,3- <i>bis</i> (β -Chloroethylthio)propane*	53	bp ^{0.02}	101°	53	34, 54

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

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TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
122. 2,2-bis(β -Chloroethylthio)propane*	53	bp ^{0.003}	65°	53	34, 54
	...	mp	-15°	53	...
123. 2-Chloro-1,3-bis(β -chloroethylthio)propane	8, 59			...	34, 18, 54, 179
124. 2,3-bis(β -Chloroethylmercapto)propanol	59			...	34, 54
125. 1,2-bis(β -Chloropropylthio)ethane*	...			...	...
126. 1-(β -Chloroethylthio)-2-butoxyethane*	...			...	243c
127. 1-(β -Chloroethylthio)-2-butylthioethane*	...			...	243c
128. 1,3-bis(β -Chloroethylthio)butane	53	bp ^{0.016}	108°	53	34, 54
129. 1,4-bis(β -Chloroethylthio)butane*	...			...	...
130. 2,3-bis(β -Chloroethylthio)butane*	59			...	34, 54
131. 1,5-bis(β -Chloroethylthio)pentane*	...			...	...
132. 1,6-bis(β -Chloroethylthio)hexane*	...			...	...
133. 1,5-bis(β -Chloropropylthio)pentane*	...			...	...
134. α, α' -bis(β -Chloroethylthio)toluene*	...			...	...
135. 1,8-bis(β -Chloroethylthio)octane*	...			...	...
136. α, α' -bis(β -Chloroethylmercapto)- <i>p</i> -xylene	59	mp	75.5-77°	59	56
137. 1,9-bis(β -Chloroethylthio)nonane*	...			...	...
138. 1,10-bis(β -Chloroethylthio)decane*	...			...	...
139. 1,2-Diphenyl-1,2-bis(β -chloroethylthio)ethane*	...			...	...
140. 9,10-bis(β -Chloroethylthio)stearic acid*	...			...	...
141. bis(β -Fluoroethylthiomethyl) sulfide*	...	bp ¹⁷	139.5°	238b	238b
142. bis(β -Chloroethylthiomethyl) sulfide*	...	bp ²⁰	125°	247	247
143. tris(β -Chloroethylthio)methane	53	bp ^{0.003}	35-40°	53	34, 54
144. β -(β -Chloroethylthio)ethyl- β' -(β -chloroethoxy)-ethyl sulfide*	...			...	...
145. bis[β -(β -Chloroethylthio)ethyl] ether (T)	See text	bp ²	174°	247	See text
	...	<i>d</i> ²⁰	1.2445	247	...
146. bis[β -(β -Cyanoethylthio)ethyl] ether*	...			...	...
147. bis[β -(β -Chloroethylthio)ethyl] sulfide* (di H)	53	mp	73-75°	53	...
148. bis[β -(β -Bromoethylthio)ethyl] sulfide*	...			...	...
149. bis[β -(β -Thiocyanoethylthio)ethyl] ether*	...			...	...
150. bis[β -(β -Phenoxyethylthio)ethyl] sulfide*	...			...	...
151. bis[β -(β -(2,4,6-Tribromophenoxy)ethylthio)-ethyl] sulfide*	...			...	...
152. 1,2,3-tris(β -Chloroethylthio) propane*	...			...	...
153. bis[β -(β -Chloropropylthio)ethyl] ether*	...			...	...
154. bis[α -Methyl- β -(β -chloroethylthio)ethyl] ether	...			...	34, 54
155. bis[β -(β -Chloropropylthio)ethyl] sulfide*	...			...	...
156. bis[β -(β -Chloroethylthio)propyl] sulfide*	...			...	...
157. 1,1,1-tris(β -Chloroethylthiomethyl) ethane	53	bp ^{5x10³-5x10⁻⁴}	41-42°	53	34
158. bis[α -Methyl- β -(β -chloropropylthio)ethyl] ether*	...			...	...
159. bis[β -(β -Chloropropylthio)propyl] sulfide*	...			...	...
160. 1,2-bis(β -(β -Chloroethylthio)ethylthio) ethane*	...			...	...
161. 1,1,2,2-tetrakis(β -Chloroethylthio) ethane	63	bp ^{0.01}	40°	63	34, 54
	...	<i>n</i> _D ^{21.5}	1.5655	63	...
162. tetrakis(β -Chloroethylthiomethyl) methane	53			...	34
163. bis[β -{ β -(β -Chloroethylthio)ethylthio}ethyl] ether*	...			...	...
164. bis[β -{ β -(β -Chloroethylthio)ethoxy}ethyl] sulfide*	...			...	...
165. bis[β -{ β -(β -(β -Chloroethylthio)ethoxy)ethylthio}ethyl] ether*	...			...	...
166. bis[β -{ β -(β -(β -(β -Chloroethylthio)ethoxy)ethylthio)ethoxy}ethyl] sulfide*	...			...	...
167. bis[β -[β -{ β -(β -(β -Chloroethylthio)ethoxy)-ethylthio]ethoxy}ethylthio]ethyl] ether*	...			...	...

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

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TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
168. β -Oximinoethyl ethyl sulfide*	...			...	...
169. β -(β -Chloroethylthio)ethyltrimethylammonium chloride	...			...	2
170. <i>p</i> -Aminophenyl- β -(β -chloroethylthio)ethylamine hydrochloride*	...			...	...
171. N- β -Chloroethylthiomorpholine*	16	bp ^{0.5}	77–80°	16	34
172. N- β -Chloroethylthiomorpholine hydrochloride	16	mp	208–209°	16	...
173. N- β -Hydroxyethylthiomorpholine hydrochloride	16	mp	160–163°	16	...
174. <i>bis</i> (β -Oximinoethyl) sulfide*	...			...	...
175. <i>bis</i> [β -(<i>bis</i> (β -Chloroethyl)amino)ethyl] sulfide*	26	mp	24.2–24.7°	26	34
	...	<i>n</i> _D ²⁵	1.5287	26	...
176. Methyl- <i>bis</i> (β -ethylthioethyl)amine	...			...	241f
177. Methyl- <i>bis</i> (β -(β -chloroethylthio)ethyl)amine hydrochloride	36	mp	70–72°	36	34
178. Ethyl- <i>bis</i> (β -(β -chloroethylthio)ethyl)amine hydrochloride	39	mp	62–64°	39	34
179. N,N- <i>bis</i> (β -(β -Chloroethylthio)ethyl)aniline hydrochloride	36	mp	69–71°	36	34
180. <i>tris</i> (β -(β -Chloroethylthio)ethyl)amine	181	mp	52°	181	181
181. α -Chloromethylthiophene	8	bp ¹³	71–76°	8	...
182. ω -Chloroacetylthiophene*	...			...	...
183. ω -Bromoacetylthiophene*	...			...	...
184. 2-Chloroacetyl-5-nitrothiophene*	...			...	...
185. Diphenyl- α -thienylstibine*	...			...	...
186. Phenylldithienylstibine*	...			...	...
187. 2-Chloromercurithiophene*	44	mp	183–184°	44	34
188. 2,5- <i>bis</i> (Chloromercuri)thiophene*	...			...	...
189. 2-(<i>p</i> -Aminophenyl)6-methylbenzthiazole*	...			...	...
190. N- β -Chloroethylphenothiazine	61a			...	...
191. Dimethyl trisulfide	53	bp ²⁰	64°	53	34
192. Dimethyl tetrasulfide	53	bp ¹	56–69°	53	34
	...	<i>n</i> _D ²⁰	1.6621	53	...
	...	<i>d</i> ²⁵	1.3008	53	...
193. <i>bis</i> (β -Chloroethyl) disulfide*	8	bp ³	80°	8	...
194. <i>bis</i> (α -Chloroethyl) trisulfide*	...			...	...
195. <i>bis</i> (β -Chloroethyl) trisulfide*	35	mp	30.5–31.5°	35	34, 54
196. <i>bis</i> (β -Chloroethyl) pentasulfide	35	<i>n</i> _D ²⁰	1.6753	35	34, 54
197. <i>bis</i> (1,3-Dichloroisopropyl) disulfide	...			...	54
198. <i>bis</i> (2-Aminophenyl) disulfide	...			...	...
199. <i>bis</i> (α -Chloromethyl) sulfoxide*	...			...	...
200. Divinyl sulfoxide*	...			...	34, 54
201. β -Chloroethyl vinyl sulfoxide	...			...	34, 54
202. <i>bis</i> (β -Chloroethyl) sulfoxide*	8	mp	108–110°	8	34
203. Thiodiglycol sulfoxide	65a	mp	110°	65a	54
204. 2,5 (or 1,3) Dihydrothiophene sulfoxide	...			...	...
205. <i>bis</i> -Sulfoxide of 1,2- <i>bis</i> (β -chloroethylthio)ethane (2 isomers)	53	mp	148°	53	34, 54
206. <i>bis</i> -Sulfoxide of 1,2- <i>bis</i> (β -hydroxyethylthio)-ethane (mixture of isomers)	65c	mp	90–101°	65c	34
207. <i>bis</i> (Ethoxyethyl) sulfoxide*	...			...	...
208. <i>bis</i> -Sulfoxide of <i>bis</i> (β -(β -chloroethylthio)ethyl) ether (2 isomers)	51	mp	100–101°	51	56
	...	mp	106–107°	51	...
209. mono-Sulfoxide of <i>bis</i> (β -(β -chloroethylthio)ethyl) sulfide*	...			...	...
210. Diphenyl sulfoxide*	...			...	...
211. <i>bis</i> (α -Chloromethyl) sulfone*	...			...	...
212. Methyl vinyl sulfone*	...			...	...
213. Methyl β -chloroethyl sulfone*	...			...	...

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

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TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
214. Divinyl sulfone*	53			...	34, 54
215. β -Bromovinyl vinyl sulfone*	...	bp ²²	137°	197	197, 241d
216. <i>bis</i> (β -Bromovinyl) sulfone*	...	mp	58-59°	241d	197, 241d
217. Ethyl vinyl sulfone*	...			...	...
218. β -Chloroethyl vinyl sulfone	53	bp ¹⁷	152-154°	53	34, 54
219. β -Chloroethyl ethyl sulfone*	...			...	...
220. <i>bis</i> (β -Chloroethyl) sulfone* (H sulfone)	53	bp ³	155°	53	34, 54
	...	mp	51.5-52.5°	53	...
221. <i>bis</i> (β -Bromoethyl) sulfone*	...			...	...
222. β -Chloroethyl- α' , β' -dibromoethyl sulfone*	...	mp	63-64°	197	197, 241d
223. <i>bis</i> (Dibromoethyl) sulfone (2 isomers)	...	mp	138°	241d	197, 241d
	...	mp	72-73°	241d	...
224. Thiodiglycol sulfone	65a	mp	56°	65a	34, 54
225. 2,5 (or 1,3) Dihydrothiophene sulfone*	...			...	...
226. 2-Chlorotetrahydrothiophene sulfone*	...			...	...
227. N- β -Chloroethylthiazan sulfone*	...			...	...
228. N- β -Chloroethylthiazan sulfone hydrochloride*	...			...	...
229. N- β -Hydroxyethylthiazan sulfone hydrochloride*	...			...	...
230. Diallyl sulfone*	...			...	...
231. <i>bis</i> (γ -Chloropropyl) sulfone*	...	mp	64-65°	197	197, 241d
232. Phenyl chloromethyl sulfone*	...			...	...
233. Phenyl vinyl sulfone*	...			...	...
234. β -Chloroethyl phenyl sulfone*	...			...	...
235. β -Chloroethyl <i>p</i> -nitrophenyl sulfone	...			...	34, 54
236. β -Chloroethyl 2,4-dinitrophenyl sulfone	...			...	...
237. β -Chloroethyl <i>p</i> -tolyl sulfone*	...			...	...
238. Diphenyl sulfone*	...			...	...
239. <i>bis</i> (2-Chlorocyclohexyl) sulfone*	...			...	...
240. <i>p</i> -Thioxane sulfone*	65b			...	34
241. <i>bis</i> -Sulfone of <i>bis</i> (β -chloroethylthio)methane*	...			...	...
242. <i>bis</i> -Sulfone of 1,2- <i>bis</i> (vinylthio)ethane*	...			...	...
243. <i>bis</i> -Sulfone of 1,2- <i>bis</i> (β -chloroethylthio)ethane*	53	mp	202-204°	53	34
244. <i>bis</i> -Sulfone of 1,2- <i>bis</i> (β -hydroxyethylthio)ethane	65c	mp	113-115°	65c	34
245. <i>bis</i> -Sulfone of 1,4- <i>bis</i> (β -chloroethylthio)butane*	...			...	...
246. <i>bis</i> -(Methoxyethyl) sulfone*	...			...	...
247. <i>bis</i> -(Ethoxyethyl) sulfone*	...			...	...
248. <i>bis</i> -Sulfone of <i>bis</i> (β -(β -chloroethylthio)ethyl) ether	51	mp	70-71°	51	56
249. <i>bis</i> (β -(β -Chloroethylthio)ethyl) sulfone*	...			...	...
250. <i>bis</i> (β -Isoamyloxyethyl) sulfone*	...			...	...
251. β -Fluoroethyl thiolacetate	59	bp ¹⁰⁰	85-87°	59	34
	...	vol ²⁰	21.4	38	...
252. β -Chloroethyl thiolacetate	...			...	34, 54
253. Diethylthallium thioacetate	19	mp	181-183°	19	...
254. Triethyllead thioacetate*	241	mp	44°	241	...
255. S- β -Chloroethyl fluorothiolacetate	55	bp ¹⁰	80-81°	55	34, 238b
256. Phenyl fluorothiolacetate	241g	mp	36.5-37.5°	241g	238a
	...	bp ¹⁸	132°	241g	...
257. β -Chloroethyl chlorothiolacetate*	...			...	...
258. Chloroacetyl thiophenol*	...			...	...
259. <i>bis</i> (Chloroacetyl) sulfide*	...			...	...
260. β -Chloroethyl bromothiolacetate*	...			...	...
261. Methyl γ -fluorothiolbutyrate	33, 55	bp ⁶	54°	33	34
	...	n_D^{20}	1.4587	33	...
	...	d^{20}	1.1135	33	...
	...	vol ²⁵	8.44	33	...
262. Methyl γ -fluoro- β -hydroxythiolbutyrate	55	bp ^{0.2}	68-71°	55	34
	...	n_D^{20}	1.4872	55	...

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
263. <i>bis</i> (β -Chloroethyl) dithioloxalate	53	mp	43°	53	34, 54
	...	bp ¹	153–160°	53	...
264. Diethyllead dithioacetate*	241	mp	84.5–85°	241	...
265. β -Chloroethyl thiolcarbonyl chloride	64b	bp	ca. 150°	64b	34
266. Reaction product of thiophosgene and β -chloro-ethylmercaptan	53			...	34, 54
267. β -Fluoroethyl xanthate*	...	mp	208–210°	238a	238a
268. β -Xanthylethyltrimethyl ammonium iodide*	...			...	...
269. Methyl N-ethylthiolcarbamate	44	bp ²⁹	118°	44	34
	...	bp ³⁹	123°	44	...
	...	d_{25}^{25}	1.078	44	...
	...	$n_D^{29.5}$	1.4978	44	...
270. Methyl N-ethylthionocarbamate	44	bp ²⁵	109.5–110.5°	44	34
	...	bp ³⁷	119–121.5°	44	...
	...	d_{25}^{25}	1.067	44	...
	...	n_D^{25}	1.5150	44	...
271. β -(Dimethylamino)ethyl N-methylthiocarbamate hydrochloride*	...			...	238c
272. β -(Dimethylamino)ethyl N-methylthiocarbamate methiodide*	...			...	238c
273. α -Naphthylthiourea	...	mp	198°	263	34
274. Methyl N-ethyldithiocarbamate	44	bp ^{1.8}	121–122°	44	34
	...	n_D^{27}	1.6139	44	...
	...	d_{25}^{25}	1.151	44	...
275. Thallous N-methyldithiocarbamate	19			...	...
276. Thallous N,N-dimethyldithiocarbamate	19	mp	124–125°	19	...
277. Thallous N-ethyldithiocarbamate	...			...	...
278. Thallous N-isopropyldithiocarbamate	...			...	...
279. Sodium N,N-diethyldithiocarbamate*	...			...	...
280. Thallous N,N-diethyldithiocarbamate	19	bp ^{0.01–0.02}	190°	19	...
	...	mp	110–111°	19	...
281. Dimethylthallium N,N-diethyldithiocarbamate	19	bp ¹	130°	19	34
	...	bp ⁴	138°	19	...
282. Sodium N,N- <i>bis</i> (β -hydroxyethyl)dithiocarbamate*	...			...	...
283. Thallous N-butyldithiocarbamate	19			...	...
284. Thallous N,N-diisopropyldithiocarbamate	19			...	...
285. Dimethylthallium N,N-diisopropyldithiocarbamate	19	bp ¹	130°	19	34, 54
	...	bp ^{5.5}	145°	19	...
	...	mp	150°	19	...
286. Thallous N-cyclohexyldithiocarbamate	19			...	...
287. Thallous N,N-dibutyldithiocarbamate	19	bp ^{0.01–0.02}	230–235°	19	...
	...	mp	75–77°	19	...
288. Dimethylthallium N,N-dibutyldithiocarbamate	19	bp ^{0.5}	147–148°	19	...
289. Thallous N,N-diisobutyldithiocarbamate	19	mp	165–165.5°	19	...
290. Dimethylthallium N,N-diisobutyldithiocarbamate	19	bp ^{0.5}	104–105°	19	34
	...	mp	73–74°	19	...
291. <i>bis</i> (β -Chloroethyl) trithiocarbonate	53	bp ²	85°	53	34
	...	n_D^{20}	1.5505	53	...
292. β -Chloroethyldimethylsulfonium chloride	23	mp	147–148°	23	34
293. β -Hydroxyethyldimethylsulfonium chloride	23			...	...
294. Methyl- <i>bis</i> (β -hydroxyethyl) sulfonium chloride	23			...	...
295. Methyl- <i>bis</i> -2-hydroxyethyl sulfonium iodide	23			...	...
296. Dithiane monomethiodide	61g	mp	168°	61g	34
297. <i>tris</i> (β -Chloroethyl) sulfonium chloride	46			...	34
298. S,S- <i>endo</i> -Ethylenedithiane sulfonium dichloride	46			...	...
299. S-Vinyldithiane sulfonium chloride	46			...	...

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

TABLE 3 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
300. S- β -Chloroethyldithiane sulfonium chloride	46	mp	144°	46	34
301. S- β -Hydroxyethyldithiane sulfonium chloride	...	mp	176°	197, 241d	197, 241d
302. Sulfonium salt of thiodiglycol and <i>bis</i> (β -chloroethyl) sulfoxide	...			...	34
303. Sulfonium salt of thiodiglycol <i>bis</i> (β -chloroethyl) sulfone	...			...	34, 54
304. Sulfonium compound of <i>bis</i> (2-chloroethyl) sulfide and 2 moles of thiodiglycol	8	mp	102–103°	8	34
305. Triethyllead cyclohexylsulfinate	20	mp	132–134°	20	...
306. Triethyllead <i>p</i> -toluenesulfinate	20	mp	86–88°	20	...
307. Methanesulphonyl fluoride*	...			...	...
308. Methanesulphonyl chloride*	...			...	...
309. Chloromethanesulphonyl chloride	...			...	...
310. Trichloromethanesulfonyl chloride*	11			...	241e
311. Ethanesulfonyl chloride	11	bp ²⁰	73.5–75°	11	34
	...	<i>d</i> ₄ ²⁰	1.370	11	...
312. 2-Fluoroethanesulphonyl chloride*	...			...	...
313. β -Chloroethylsulphonyl chloride*	...			...	...
314. β -Bromoethanesulfonyl fluoride	64d	bp ²⁰	90°	64d	...
315. Chloropropanesulfonyl chloride	Commercial			...	34
316. Butanesulfonyl fluoride	64c	bp ⁸	54–56°	64c	34
317. Butanesulfonyl chloride	64c	bp ⁷	76–78°	64c	34
318. Ammonium 2-chloroethanesulfonate	...			...	34
319. Sodium γ -fluoro- β -hydroxy propanesulfonate	55			...	56
320. Cadmium <i>m</i> -nitrobenzenesulfonate	9			...	...
321. Lead <i>m</i> -nitrobenzenesulfonate	3			...	...
322. Cadmium 2,4-dinitrobenzenesulfonate	9			...	...
323. Lead 2,4-dinitrobenzenesulfonate	3			...	...
324. Triethyllead <i>o</i> -toluenesulfonate*	...			...	...
325. Tripropyllead <i>o</i> -toluenesulfonate	241	mp	87°	241	...
326. Trimethyllead <i>p</i> -toluenesulfonate*	...			...	...
327. Triethyllead <i>p</i> -toluenesulfonate*	...			...	...
328. Tripropyllead <i>p</i> -toluenesulfonate	241	mp	73–74.5°	241	...
329. Tributyllead <i>p</i> -toluenesulfonate	241	mp	81–82°	241	...
330. Triethyllead 2-amino-5-toluenesulfonate	20	mp	210°	20	...
331. Triethyllead naphthalene-2-sulfonate*	...			...	...
332. Tributyllead naphthalene-2-sulfonate*	241	mp	68°	241	...
333. Triethyllead 1-amino-4-naphthalenesulfonate	20	mp	238–240°	20	...
334. Dipropylthallium <i>d</i> -camphor-10-sulfonate	19			...	...
335. Triethyllead <i>d</i> -camphor-10-sulfonate	20	mp	172°	20	...
336. Triethyllead <i>p</i> -tolylthiosulfonate	20	mp	109°	20	...
337. <i>bis</i> -Triethyllead methanedisulfonate	241b			...	241b
338. Triethyllead methanesulfonamide*	241b	mp	97°	241b	241b
339. Tripropyllead methanesulfonamide*	241b	mp	67°	241b	241b
340. Triethyllead methanesulfonanilide*	241b	mp	115.5°	241b	241b
341. <i>bis</i> -Triethyllead methanedisulfonanilide	241b			...	241b
342. Triethyllead ethenesulfonanilide	241b	mp	110°	241b	241b
343. Triphenyltin benzenesulfonamide	241b	mp	119°	241b	241b
344. Triethyllead benzenesulfonamide	...			...	241c
345. Tripropyllead benzenesulfonamide	...			...	241c
346. Triethyllead <i>p</i> -aminobenzenesulfonamide*	20, 241b	mp	173–174°	20	241b
347. Tripropyllead <i>p</i> -aminobenzenesulfonamide	241b	mp	101°	241b	241b
348. Triethyllead <i>o</i> -toluenesulfonamide*	241b	mp	133°	241b	...
349. Triethyllead <i>p</i> -toluenesulfonamide*	...			...	241c
350. Triethyllead <i>p</i> -toluenesulfonanilide*	241b	mp	134°	241b	...
351. Tripropyllead <i>p</i> -toluenesulfonanilide	241b	mp	104°	241b	241c
352. Triethyllead <i>p</i> -toluenesulfon- <i>p</i> -chloranilide	241b	mp	111.5°	241b	241b
353. Triethyllead <i>p</i> -toluenesulfon- <i>p</i> -bromanilide	241b	mp	117°	241b	241b
354. Tripropyllead <i>p</i> -toluenesulfon- <i>p</i> -chloranilide*	241b	mp	123°	241b	241b
355. Triethyllead <i>o</i> -carboxybenzenesulfonimide	241b	mp	135°	241b	...
356. Tripropyllead <i>o</i> -carboxybenzenesulfonimide	241b	mp	130°	241b	241b

* Not all the British reports concerning compounds marked with an asterisk are available in this country. References are contained in reference 176.

The high freezing point of H is a disadvantage in some instances. For aircraft spray tanks, in particular, there was interest in a vesicant mixture which would withstand high-altitude temperatures of -30 to -40 C for several hours without freezing. This degree of lowering of the freezing point could be attained only by the use of a relatively large percentage of an inert diluent such as acetone or benzene. Such mixtures suffered from the relatively low payload of active vesicant agent. The freezing point could be lowered a small amount by mixture with Q or T. The freezing points of eutectic mixtures of H with pure Q or T are as follows: HQ (68/32) 4.5 C and HT (35/65) approximately -8 C.²¹³

The principal U. S. standard charging was H (Levinstein), which with its 30 per cent nonvolatile impurities melted at about 8 C. The standard British chargings included HT (60/40) melting at about 0 C and solutions of H containing 10–20 per cent benzene.

The viscosity of H is an important property not included in the above tabulation. The size of drops of H from spray munitions is a function of the resistance of the charging to shattering forces upon emission and this property is related to the viscosity of the material. The addition of "thickening" agents, such as methacrylate polymers, was first studied from the standpoint of the use of H in high-altitude aircraft spray, where large drops were essential to prevent a high percentage of evaporation before the charging reached the ground. Also, the toxicological data showed that large drops of H are more efficient than small ones in producing vesication by direct contact, particularly against personnel in permeable protective clothing. Since high-altitude spray was not adopted as a practical procedure, partly because of the inherent inaccuracies in aiming, and since the primary interest in H became based on vapor effects rather than liquid contact, thickened chargings were not adopted for standard U. S. munitions. The methods for their preparation, however, were carefully worked out and field trials performed.¹⁷³ The NDRC research on the preparation and physical properties of thickened H chargings was carried out by Division 11.

5.4.2 Detection and Analysis

Chemical methods for the detection and analysis of H are outlined in Chapter 34. The introduction of 4-(*p*-nitrobenzyl)pyridine (DB-3) as a colorimetric

agent for β -chloroethylthio compounds greatly simplified the detection problem. The development of continuous recording titrimeters for the measurement of H concentrations led to the wide use of these instruments in U. S. and British field trials and as integral parts of gassing chambers for toxicological measurements.

H is detectable by odor at about 0.0006 mg/l.¹¹² Field experience showed that a sulfide odor from Levinstein H residues can persist after the active H has evaporated. The instructions for troops required the wearing of masks whenever H could be detected by odor, unless subsequent careful gas reconnaissance with DB-3 tubes established the fact that the odor was from nontoxic residues, the DB-3 test being specific for the active agent.

The researches on protective clothing, protective ointments, and decontamination procedures for H and related vesicants are covered in Part IV.

5.5

TOXICOLOGY

5.5.1

Vesicancy Tests

The action of the vesicant agents on the skin is their most important toxicological property. Since World War I extensive data have been accumulated on liquid agents tested by application of droplets to the bare skin of the forearm. Since the vesicant agents can be adequately assessed only against human skin, the availability of volunteers has been of major importance in this program.

The performance of skin tests under conditions which permit quantitative interpretation of the results requires that the testing procedure be carefully controlled. The introduction of specially designed micropipets (see Chapter 16) has facilitated the routine application of very small volumes (0.01 mm³ or less).^{7,37,54} Evaporation is a variable entering into tests with volatile agents. The term "absolute" vesicancy has been introduced by British investigators to indicate the activity of the compound when it is applied to the skin and then covered to prevent evaporation. For example, in the usual "open" vesication test, Q is many times more potent than H. Under conditions of "absolute" or "closed" vesication, which is important in the study of the intrinsic activity of different molecular structures, the two agents are much nearer to each other in activity.

For the study of the vesicancy of the vapor of an agent, rather than that of the liquid in contact with

TABLE 4. Relative vesicancies of H, Q, and T.

Compound	Uncovered	Reference	Vesicancy relative to H		Vapor	Reference
			Covered	Reference		
H	1	...	1	...	1	...
Q	5*	199	2	199	...	...
T	3*	199	<1	198	...	...
Methyl- β -chloroethyl sulfide	..	...	0.06	199	<0.1	30
Ethyl- β -chloroethyl sulfide	..	...	0.06	202	<0.1	30

* More recently determined and higher relative potencies based on median vesicating doses of 32 mg for H, 4 for T, and 0.3 for Q (dissolved in dioxane) are given elsewhere.⁵² (See Chapter 23.)

the skin, several techniques have been developed. The effect of the vapor on animals in body exposure experiments, as outlined in the section on $L(Ct)_{50}$'s, gives evidence that bears on the problem. The most direct and important of the experimental procedures is the use of chambers for the exposure of volunteers under conditions of temperature and humidity approximating as closely as possible field conditions of exposure¹⁷³ (see also Chapter 23). For laboratory screening of agents on human skin, however, vapor cups or other mechanisms for producing burns on small areas of the skin of the forearm have been employed. In dynamic tests, in contrast to static tests, a stream of a known concentration of the gas in air may be directed at a small area of skin for a given time.^{30,54}

The relative vesicancies of H, Q, and T are listed in Table 4. The references to the vesicancy tests on the wide variety of sulfur mustard compounds are included in Table 3. British surveys of the relationship of structure and vesicancy are included in the Bibliography.^{176,198,199,202,236} Vesicancy data are summarized and tabulated by NDRC^{17,34,48,52,54,68} (see also Chapter 23).

The data on two of the more volatile analogs of H are included in Table 4 for comparison. There would be interest in a compound as vesicant as H but more volatile. The vesicant potency of H is not approached, however, by any of the more volatile β -chloroethylthio compounds. Only among the compounds with relative low vapor pressure have more potent analogs been found. These have the advantage of greater effectiveness in contact with the bare skin but they have poorer penetration through clothing and negligible vapor action.

The laboratory tests give indications of the relative activities of the different compounds. The actual Ct 's or liquid contaminations required under field

conditions to incapacitate troops are determinable only through chamber and field trials (see Section 5.6).

5.5.2 $L(Ct)_{50}$'s of H, Q, and T

The $L(Ct)_{50}$ values for total exposure of different species are summarized in Table 5.

For agents in this class the $L(Ct)_{50}$ for the mouse or the rat serves as a useful index of the potency of agent even though final assessment may rest on vesicant action. H has an $L(Ct)_{50}$ for the mouse or the rat of about 1,000 mg min/m³ and this figure became an informal base line in the screening of new agents. Potential persistent agents showing an $L(Ct)_{50}$ near to or less than 1,000 were usually selected for more detailed study. In the case of H, it will be noted that the species variation is small. The rate of detoxification of H is known to be low in tests on human skin¹⁶⁹ and eyes^{60k,169,187} and in most of the $L(Ct)_{50}$ experiments, although the data in the case of the mouse show an increase in $L(Ct)_{50}$ with time which is not in line with this generalization. From the data on rats the calculated detoxification constant is 0.002 mg/l.⁸¹ The high toxicity of Q under laboratory conditions is a value which could be attained in the field only if equally efficient means for production of the aerosol could be achieved. The laboratory results vary with the particle size obtained in the aerosol (see Chapters 12 and 15).

Among the volatile analogs of H, none of them exceed H in toxicity. *bis*(β -Bromoethyl) sulfide has about the same $L(Ct)_{50}$ for the mouse as does H.^{60j} The methyl, ethyl,^{60f} and benzyl- β -chloroethyl sulfides, for example, are much less active. A summary of the relative toxicities of the analogs of H is given elsewhere.^{60d}

The $L(Ct)_{50}$'s tabulated in Table 5 are a function of the action of the vesicant agent on and through

TABLE 5. $L(Ct)_{50}$'s of H, Q, and T for different species.
(Exposure of the entire animal* — 15-day observation period.)

Agent	Species	Exposure times (min)	$L(Ct)_{50}$ (mg min/m ³) or suggested value where number of animals is small	A = anal. conc. N = nom. conc.	Number of animals used	Reference
H	Mouse	2	860	A	160	80
		10	1,200	A	230	50
		10	1,200	A	180	123
		60	1,380	A	140	80
		360	4,140	A	277	80
	Rat	2	840	A	50	80
		10	800	N	48	50
		10	850	A	80	80
		60	900	A	60	80
		360	1,512	A	40	80
	Guinea pig	10	1,700	N	19	50
	Rabbit	10	900	N	8	50
		ca. 30	1,025	A	80	83b
	Cat	10	700	N	10	50
	Dog	10	600	N	8	50
	Goat	10	1,900	A	60	166
	Monkey	10	800	N	3	50
Q	Mouse	10	170	A	190	50
		10	350	A	...	120
		10	270	A	200	82
	Rat	10	400	N	34	50
	Guinea pig	10	>1,600	A	26	50
	Rabbit	10	2,000	N	12	50
	Cat	10	900	N	11	50
	Dog	10	1,400	N	6	50
	Mouse	10	1,650†	A	180	18
HQ 90/10	Mouse	10	820	A	200	120
HQ 75/25	Mouse	10	770	A	240	120
HT 60/40	Mouse	10	820	A	260	123

* The toxicities in this table are for total exposure of the animals at low rates of air flow. (See Table 6.)

† The aerosol was generated under conditions different from those prevailing in the preceding tests. Under the conditions of this experiment, Q gave an $L(Ct)_{50}$ of 700.

the skin of the animal as well as on the lungs. Toxicological techniques have been worked out in detail⁴⁵ for differentiating the relative contributions of the actions of the agent on the lungs and on the body surface. In the case of agents such as T or Q, which were dispersed by atomization into the exposure chambers, the rate of air flow was an additional variable which affected the $L(Ct)_{50}$ by influencing the impingement of the aerosol particles on the skin. The most extensive studies have been made with the mouse. The results with HN1, HN3, and L are included for comparison in Table 6.

The body exposure experiments were carried out in an apparatus which exposed the body of the animal to the toxic agent while the head was in uncontaminated air. In the inhalation experiments only the head was exposed to the toxic atmosphere. The linear velocity of air flow through the chamber was

TABLE 6. Effects of flow rate and type of exposure on $L(Ct)_{50}$'s of vesicant agents for mice.*⁵⁰

Type of exposure	Degree of air flow	H	Q	HN1	HN3	L
Total	Low	1,200	170	900	590	1,400
	High	1,400	100	900	300	900
Body	Low	3,500	1,500	4,800	1,000	1,900
	High	3,400	510	3,100	370	1,200
Inhalation	Low	1,600	280	1,300	1,100	1,400
	High	1,600	250	1,300	1,200	1,500

* Exposure periods are 10 minutes except for some body exposures to HN1 and Q ranging from 5 to 45 minutes.

$\frac{1}{16}$ mph at the low flow rate and 3 mph at the high flow rate.

With H, which is present entirely in the vapor form, the $L(Ct)_{50}$ for the three types of exposure is

not significantly altered by change in flow rate. It might be anticipated that an increase in wind speed would tend to disturb the cushion of air held in the fur of an animal and increase the concentration of vapor at the surface of the skin. The experiments with HN1 give an indication of such an effect but those with H give none. In no case is the inhalation toxicity significantly affected by flow rate.

With Q, which is present as an aerosol, the body toxicity increased markedly with increase in air flow. This is also true of HN3, which is present in part as an aerosol, and of L (see Chapter 7). (The authors of Table 2 point out that the same toxicity for L by total exposure and by inhalation at low flow is an anomaly not in line with the rest of the data.)

For H, HN1, and HN3, the reciprocal of the $L(Ct)_{50}$ by total exposure corresponds fairly closely to the sum of the reciprocals of the $L(Ct)_{50}$'s by body exposure and by inhalation, respectively.⁵⁰

The body toxicities become less significant relative to the inhalation toxicities as the size of the animal increases. For the dog and the monkey the body $L(Ct)_{50}$'s are about 11,000 and 14,000 respectively.⁵⁰

It is reasonable to assume that the $L(Ct)_{50}$ of H for man by exposure which includes inhalation falls within the range of values in Table 5, namely, 1,000 to 2,000 mg min/m³. In calculations on the effectiveness of H against troops, however, in view of the adequacy of the modern gas mask, this value has logically received less consideration than those based on the production of casualties by body exposure. The power of H lies primarily in its ability to produce casualties despite the mask. In the case of man, the evidence indicates that the actual production of death by body exposure requires Ct 's of more than 10,000 in temperate weather. There are no data to establish with certainty whether the $L(Ct)_{50}$'s of H for man by body exposure fall within the range that can be attained by feasible munition expenditures. But death is not the objective which requires primary consideration in the assessment of H. Sublethal skin injuries from H are capable of totally disabling troops for periods of weeks and, as outlined in Section 5.6, sufficient data are available from tests on man to indicate the dosages required to yield different degrees of disability. Since man is a sweating animal and the effectiveness of H on skin is markedly affected by the degree of surface moisture (see Chapter 23), the results on body exposure of animals to H have no direct bearing on the estimation of casualty-producing dosages for man. The experiments on

body exposure have had a direct use in supplying animals for study of the systemic effects from absorbed H under conditions where the lungs are protected.^{83a}

5.5.3 Action of H on the Eyes

The toxicity of H vapor to the eyes is a subject of special importance. Serious injury may be produced by low dosages, and loss or impairment of vision is a casualty effect of primary significance. In addition to the $L(Ct)_{50}$ tests, the principal vesicant gases were screened for effectiveness against animal eyes.^{60d,g} In the dog corneal ulceration is produced by H at Ct 's of about 400 mg/l.^{60d} In the rabbit severe corneal opacity is produced at Ct 800 with clearly characterized ocular lesions of graded severity over the range from Ct 200 to Ct 1,200.^{94,167} This gradation was utilized by the Bushnell, Florida, installation as a practical bioassay for determining Ct values of H in field trials.¹⁶⁷

The animal tests on eyes have proved useful for preliminary tests on toxicity but tests on man have been essential for the establishment of casualty-producing dosages. British investigators have shown by chamber tests that the human eye is about four times as sensitive to H as the rabbit eye.¹⁸⁷ A Ct of 100 will cause serious impairment of vision for 24–48 hours and it is estimated that a Ct of 200 will produce temporary blindness for a week or more.

Liquid H and T and particles of the solid vesicants in the eye all produce extremely severe ocular lesions. The subject of the action of vesicants on the eye has been reviewed in detail by the Committee on Medical Research [CMR].⁶⁶ A wide variety of organic compounds was supplied by NDRC Division 9 for the studies of possible therapeutic agents for liquid vesicants in the eye.

The pathology and the physiological mechanism of action of vesicants are treated in Part III of this report. The data reviewed there together with the sections on these subjects in the reference just cited⁶⁶ give the basis for the conclusion that prompt decontamination is the only treatment of special value in mustard burns of the skin or of the eye.

5.5.4 Toxicity in Drinking Water

In connection with the program on decontamination of water supplies (Chapter 39) it has been necessary to determine the toxicities of compounds which might be produced by hydrolysis or in the course of chemical treatment. Methods based on oxidizing

TABLE 7. Toxicity in drinking water.

	Conc. in water (ppm)	Duration of administration (days)	Results on mice		Reference
			Deaths	Weight gain	
H-sulfoxide	100	30	0/30	Normal	60b
H-sulfone	100	30	0/30	Normal	60b
Sulfonium salt of H and 2 moles of thiodiglycol	100	4	13/30 (in 30 days)		60b
	100	4	6/30		60d
	100	30	6/30		60d
	50	30	0/30	Normal	60c
	25	30	0/30	Normal	60c
Sulfoxide of the above sulfonium salt	1,000	30	15/30		60b
	100	30	0/30	Normal	60b
Sulfilimine of H and chloramine-T	Sat. sol.				
	<100	30	2/30	Normal	60b
Thiodiglycol sulfoxide	1,000	28	1/30		60a
Thiodiglycol sulfone	1,000	28	0/30		60a
Q-sulfoxide	100	7	0/20		60e
			1/20		60e

agents would yield sulfoxides and sulfones. Representative data on the toxicities in drinking water of derivatives of H are given in Table 7.

For H and related β -chloroethyl vesicants the maximum safe concentration is considered to be 2 ppm, as indicated by the DB-3 test, for water to be used for a period not greater than 1 week.²⁸ In the tests on mice the sulfoxides and sulfones in this series are shown to be relatively nontoxic. The sulfonium salt of H and thiodiglycol, however, retains considerable toxicity. It reacts with DB-3 and this color test thus serves to indicate active sulfonium salts present as well as unchanged H.

LD_{50} doses of sulfur mustards by intravenous, subcutaneous, or percutaneous routes of administration are tabulated in Chapter 22.

5.6 EVALUATION AS WAR GASES

Among the more volatile vesicant agents H retains its position as the most effective war gas in this class. For special purposes the nitrogen mustards would have some uses (see Chapter 6) but, among the hundreds of analogous compounds that have been studied since H was first used in 1917, no agent has been found to have a more advantageous combination of toxicological, chemical, and physical properties.

There would have been interest in an agent as vesicant as H vapor but much more volatile. In the absence of an agent meeting these specifications, two approaches were made to the problem of increasing the rate of evaporation of H in the field. At the close of World War II, these two approaches had not

reached a stage of completion permitting final assessment of their potential usefulness. The first was the thermal generator bomb under development by Division 10 NDRC. The second approach was the addition of several per cent of a pyrogenic material, such as white phosphorus, to the H charging, an interesting modification developed by Division 11 NDRC.⁶⁰

The British, Canadian, Australian, and United States field test installations greatly extended the technical knowledge of the field behavior of H over the status of the information at the close of World War I. Division 9 NDRC was a participating agency throughout the studies on H munitions at the Chemical Warfare Service Mobile Field Unit operating at Bushnell, Florida, during 1944 and 1945. The results of this extensive program, which was one of the most important phases of chemical warfare research, have been thoroughly summarized in the formal reports from Dugway Proving Ground and elsewhere.¹⁷³

The chamber trials on H carried out on human volunteers in conjunction with the research programs of the field installations have provided documentation for the estimation of the casualty-producing power of H vapor on man. Table 8¹⁷³ summarizes the quantitative information on the amounts of H vapor required to produce physiological effects of military significance.

The nonvolatile vesicants Q and T, in mixture with H, possess the advantage of providing a contamination of ground and matériel which would remain a potential contact hazard (but not a vapor hazard) for days under meteorological conditions where H

TABLE 8. Dosages of H vapor for production of injuries in man.¹⁷³

Protection	Effect	H dosage (mg min/m ³)			Disability	Time of onset	Duration
		Hot and humid weather, temp. above 80 F, sweating skin	Warm weather, temp. 60–80 F, skin not wet with sweat	Cool weather, temp. 40–60 F, cool, dry skin			
None (No mask or protective clothing)	No significant injury; maximum safe dosage	50	50	50			
	Eye damage of threshold military significance	100	100	100	Partial	6–24 hr	1–3 days
	Temporary blindness	200	200	200	Total	3–12 hr	2–7 days
Mask (No protective clothing)	No significant injury; maximum safe dosage	100	150	400			
	Skin burns of threshold military significance	200	300	1,000	Partial	2–12 days	1–2 weeks
	Severe genital burns	500	1,000	2,000–4,000	Partial	2–7 days	1–4 weeks
	Severe generalized burns	750	2,000–4,000	4,000–10,000	Total (Partial)	About 24 hr (4–12 hr)	1–2 weeks (3–6 weeks)

would persist for a number of hours. In contact with the bare skin, Q is the most powerful vesicant known. The disadvantages of mixing Q with H relate chiefly

to the use against a target which it is desired to occupy quickly, since the presence of Q would present a persistent hazard to the occupying troops.⁶⁸

Chapter 6

NITROGEN MUSTARDS^a

By Arthur C. Cope, Marshall Gates, and Birdsey Renshaw

6.1 INTRODUCTION

DURING THE 1930's the synthesis of various tertiary *bis*(β -chloroethyl)amines, now called nitrogen mustards, was described in the open literature and references made to their vesicant actions. As a consequence, these substances were investigated by the chemical warfare services of most or all nations before and during World War II. The nitrogen mustards that were found to merit the most serious consideration were ethyl-*bis*(β -chloroethyl)amine (HN1), methyl-*bis*(β -chloroethyl)amine (HN2), *tris*(β -chloroethyl)amine (HN3), and isopropyl-*bis*(β -chloroethyl)amine. The toxicity, vesicancy, eye-injuring action, potential effectiveness as water poisons, relative lack of odor, order of volatility, and low freezing point of these compounds made them potential competitors of the standard persistent agent, mustard gas, *bis*(β -chloroethyl) sulfide (H).

HN2 is not now seriously considered for use as a war gas because of the degree of instability it has been found to possess, and isopropyl-*bis*(β -chloroethyl)amine is disqualified because its toxicological potencies are somewhat inferior to those of HN1 and HN3.

HN1 and HN3 remain as potential substitute agents for H. Although they are not believed to possess the general utility of H, they may be of value under special circumstances. In particular, HN3 would seem to be admirably suited for use in high explosive-chemical shell, and it was the intention of the German Army to use it in this way in the event of chemical warfare. In so far as classical chemical warfare continues to be of military importance, the present reviewers believe that this method of employing HN3 merits careful consideration from both the offensive and defensive points of view.

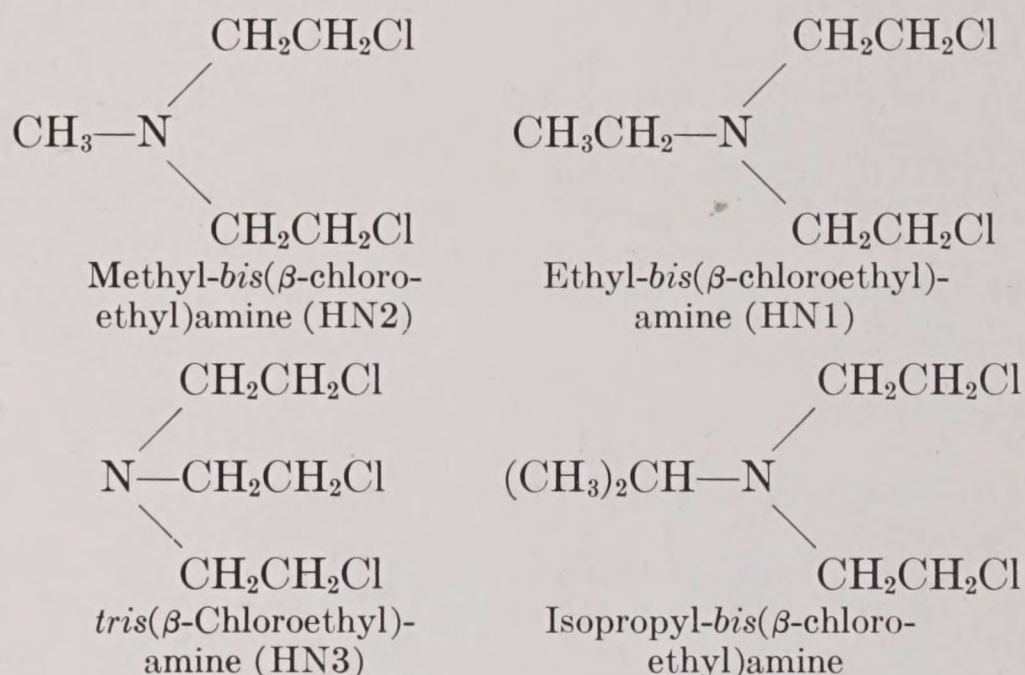
This chapter is not in itself a complete review of all available information relating to the value of the nitrogen mustards as chemical warfare agents. It should be read as a supplement to the several previous assessments that are already avail-

able.^{112,115,118,153,156} No attempt is made to duplicate the chemical and physiological phases of the subject that are covered in detail in Chapters 19 to 23.

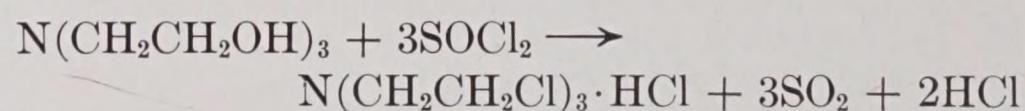
6.2 SYNTHESIS AND PROPERTIES

6.2.1 Synthesis

Many nitrogen mustards and related compounds were prepared during World War II for evaluation as possible chemical warfare agents (see Table 1). The following four tertiary *bis*(β -chloroethyl)amines proved to have the greatest practical importance and were studied most intensively.



The most practical method for preparing all compounds of this class is from the corresponding hydroxy compounds (ethanolamines) and thionyl chloride, e.g.,



The free amines or their hydrochlorides may be used in the reaction, which is conducted either in a solvent such as ethylene chloride or without a solvent. The resulting hydrochloride salts are converted to the free bases with aqueous sodium hydroxide. This method for preparing HN3 is described in the open literature.^{201,202,203,205} Similar preparations of HN2 are also described.^{198,204,206,207}

The application of the method to the preparation of the large series of homologs listed in Table 1 is discussed elsewhere.^{2,4,5,7,9,10,11,16,23,32,83,120,136,157,158,163,179a}

^a Based on information available to Division 9 of the National Defense Research Committee [NDRC] as of March 1, 1946.

TABLE 1. Nitrogen mustards and related compounds examined as candidate chemical warfare agents.

The compounds are arranged in four large classes: (1) derivatives of primary amines, (2) derivatives of secondary amines, (3) derivatives of tertiary amines, and (4) quaternary ammonium salts. Within the first two, the arrangement is in order of increasing number of carbon atoms attached to nitrogen, with acyl and other derivatives listed under the parent amines. In the last two, the arrangement is in order of increasing number of carbon atoms attached to nitrogen. Heterocyclic compounds are not segregated.

The following abbreviations are used: n_D^t , refractive index at t C; d^t , density in g/ml at t C; $d_{t_2}^{t_1}$, specific gravity at t_1 C in reference to water at t_2 C; mp, melting point in C; bp^p, boiling point in C at p mm Hg; vp^t, vapor pressure in mm Hg at t C; and vol^t, saturation concentration (volatility) in mg/l at t C.

British reports describing the examination of compounds marked with an asterisk are not all available.

Centigrade scale is used throughout the table.

Compound	Reference to synthesis	Physical properties			Reference to toxicity and vesicancy data
		Property		Reference	
A. Derivatives of primary amines					
1. β -Chloroethylamine	11	bp ^{0.5}	10–20°	11	...
2. β -Chloroethylformamide	7	bp ^{0.3}	93–96°	7	...
3. β -Chloroethyl-N-nitrosoformamide	7	bp ^{0.8}	78–80°	7	...
4. N- β -Chloroethylacetamide	7	bp ^{2.3}	107–111°	7	...
5. N- β -Chloroethyl-N-nitrosoacetamide	7	bp ^{0.5}	70–72°	7	29, 41
6. N- β -Chloroethylfluoroacetamide	60c, 178	bp ^{0.3}	77°	178	29
	...	mp	65°	178	...
7. N- β -Chloroethylchloroacetamide*	7	mp	54°	7	...
8. N- β -Chloroethyltrichloroacetamide	7	mp	75°	7	...
9. Ethyl N- β -chloroethyloxamate	7	mp	68°	7	...
10. Methyl N- β -chloroethylcarbamate	7	n_D^{20}	1.4575	61	29, 134
	...	bp ¹⁴	100°	7	...
11. Methyl N- β -chloroethyl-N-nitrosocarbamate	7, 116, 124, 162, 55	n_D^{19}	1.4666	116	29, 41, 116, 124
	...	d^{25}	1.2053	116	...
	...	bp ^{0.5}	72–76°	7	...
	...	vol ²⁰	0.600	31	...
12. Methyl N- β -chloroethyl-N-nitrocarbamate	7	bp ^{0.3}	95–100°	7	29, 41
	...	vol ²⁰	0.138	31	...
13. Ethyl N- β -chloroethylcarbamate	7	bp ¹⁴	100°	7	...
14. Ethyl N- β -chloroethyl-N-nitrosocarbamate	7	bp ¹⁴	92–93°	7	29, 41
	...	vol ²⁰	0.426	31	...
15. β -Fluoroethyl N- β -chloroethylcarbamate	53a	bp ^{0.5}	105–108°	53a	...
16. β -Fluoroethyl N- β -chloroethyl-N-nitrosocarba- mate	53a	bp ²	118–121°	53a	29
17. β -Chloroethyl N- β -chloroethyl-N-nitrosocarba- mate	7	bp ¹	120°	7	...
18. Isopropyl N- β -chloroethyl-N-nitrosocarbamate	7	bp ^{0.5}	80°	7	29, 41
19. Butyl N- β -chloroethyl-N-nitrosocarbamate	7	bp ^{0.6}	95°	7	29, 41
20. <i>sym-bis</i> (β -Chloroethyl)urea	7	mp	125°	7	...
21. N- β -Chloroethylaminophosphoryl chloride	53g	bp ¹	146°	53g	29
22. Sodium salt of N-nitro- β -chloroethylamine	50	...		...	...
23. β -Chloroethylisocyanate	53c	bp	135–137°	60b	29
24. β -Chloroethylisothiocyanate	11	mp	104°	11	...
25. β -Chloroethyl azide	21	n_D^{20}	1.4658	21	29
	...	bp ^{22.5}	38–38.5°	21	...
26. β -Bromoethyl azide	21	n_D^{20}	1.5082	21	...
	...	bp ²²	51–52°	21	...
27. β -Idoethyl azide	21	bp ²⁰	67–69°	21	...
28. β -Chloroethylisocyandichloride	11	bp ¹⁰	70–73°	11	29
29. Methyl N- β -bromoethyl-N-nitrosocarbamate	53g, 182c	bp ¹	110–115°	53g	41, 134
30. N-Bromoethylphthalimide	...	...		...	...
31. β, β, β -Trifluoroethylamine	...	...		...	29
32. β, β, β -Trifluoroisopropylamine	...	...		...	29
33. Methyl N- β -chloropropylcarbamate	7	bp ²⁰	101–103°	7	...
34. Methyl N- β -chloropropyl-N-nitrosocarbamate	7	bp ^{0.3–0.5}	75–80°	7	29, 41

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties			Reference to toxicity and vesicancy data
		Property		Reference	
<i>B. Derivatives of secondary amines</i>					
35. Ethylenimine*	11	bp	54–56°	11	29
36. N-Carbomethoxyethyleneimine	7	bp ¹⁴	42–45°	7	29
37. Propyleneimine	...	...		...	29
38. β -Chloroethylmethylnitrosoamine*	182b	...		...	134
39. N- β -Chloroethyl-N-methylchloroacetamide*	...	...		...	...
40. N- β -Chloroethyl-N-methylcarbamyl chloride*	...	...		...	...
41. N- β -Chloroethyl-N-methylaminophosphoryl chloride	48c	...		...	29
42. Ethyl- β -chloroethylamine	16	bp ²	10°	16	29, 41
43. Ethyl- β -chloroethylamine hydrochloride	16	mp	218–220°	16	29
44. 3,5-Dimethyl-4-nitrosophenyl N- β -chloroethyl-N- ethylcarbamate	38	mp	84–85°	38	29
45. 3,5-Dimethyl-4-nitrophenyl N- β -chloroethyl-N- ethylcarbamate	38	mp	42–45°	38	29
	...	bp ²	210–211°	38	...
46. 4-Nitrosothymyl N- β -chloroethyl-N-ethylcarba- mate	35	mp	49–50°	35	29
47. 4-Dimethylaminothymyl N- β -chloroethyl-N- ethylcarbamate methochloride	35	mp	161–162°	35	29
48. 4-Dimethylaminothymyl N- β -chloroethyl-N- ethylcarbamate methiodide	35	mp	165–165.5°	35	29
49. N- β -Chloroethyl-N-ethylaminophosphoryl chlo- ride	48d	...		...	29
50. <i>bis</i> (β -Fluoroethyl)amine*	182e	bp ⁷⁶⁴	123–126°	177e	177e
51. <i>bis</i> (β -Chloroethyl)amine*	60a	...		...	29
52. <i>bis</i> (β -Chloroethyl)chloramine	11	bp ¹⁰	78–80°	11	29, 134
53. <i>bis</i> (β -Chloroethyl)nitrosoamine*	7	Cannot be distilled		7	29, 41
54. <i>bis</i> (β -Chloroethyl)cyanamide	7	bp ^{0.5}	123–126°	7	29, 41, 134
55. Boron fluoride complex of <i>bis</i> (β -chloroethyl)amine	50	...		...	...
56. <i>bis</i> (β -Chloroethyl)methoxyamine*	...	...		...	134
57. <i>o</i> -Nitrobenzenesulfenyl- <i>bis</i> (β -chloroethyl)amine	32	mp	104–105°	32	29
58. <i>bis</i> (<i>bis</i> (β -Chloroethyl)amino) sulfide	32	mp	59.5–60°	32	29
59. <i>bis</i> (β -Chloroethyl)formamide	51b	...		...	29, 41
60. <i>bis</i> (β -Chloroethyl)carbamyl chloride	7	bp ^{0.3}	100–105°	7	29
61. N,N- <i>bis</i> (β -Chloroethyl)acetamide*	51b	...		...	29, 41
62. N,N- <i>bis</i> (β -Chloroethyl)fluoroacetamide	53f, 178	mp	64–5°	53f, 178	...
	...	bp ^{0.04}	102°	53f, 178	...
63. N,N- <i>bis</i> (β -Chloroethyl)chloroacetamide*	...	...		...	...
64. N,N- <i>bis</i> (β -Chloroethyl)trichloroacetamide	7	mp	97°	7	29, 41
65. <i>tetrakis</i> (β -Chloroethyl)urea	7	bp ²	168–172°	7	29, 41
66. Ethyl N,N- <i>bis</i> (β -chloroethyl)carbamate	...	...		...	29, 41
67. 3,5-Dimethyl-4-nitrosophenyl N,N- <i>bis</i> (β -chloro- ethyl)carbamate	38	mp	92–93°	38	29
68. 3,5-Dimethyl-4-nitrophenyl N,N- <i>bis</i> (β -chloro- ethyl)carbamate	38	mp	58–59.5°	38	29
69. 4-Nitrosothymyl N,N- <i>bis</i> (β -chloroethyl)carba- mate	35	mp	111–112°	35	29
70. 4-Nitrothymyl N,N- <i>bis</i> (β -chloroethyl)carbamate	35	mp	58–58.5°	35	29
71. 4-Dimethylaminothymyl N,N- <i>bis</i> (β -chloroethyl)- carbamate methochloride	35	mp	153–154°	35	29
72. 4-Dimethylaminothymyl N,N- <i>bis</i> (β -chloroethyl)- carbamate methiodide	35	mp	154–155°	35	29
73. N,N- <i>bis</i> (β -Chloroethyl)amino dichlorophosphine	11	bp ¹⁰	130–137°	11	29
74. N,N- <i>bis</i> (β -Chloroethyl)amino- <i>bis</i> (β -chloroethyl- thio)phosphine	48e	...		...	...
75. N,N- <i>bis</i> (β -Chloroethyl)aminophosphoryl fluoride	48e	...		...	29
76. N,N- <i>bis</i> (β -Chloroethyl)aminophosphoryl chloride	53f	mp	54°	53f	...
77. Methyl <i>bis</i> (β -chloroethyl)amidocyanophosphate	60d	...		...	106b

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity and vesicancy data
		Property			
78. Dimethyl <i>bis</i> (β -chloroethyl)amidophosphate	60d	...		...	106b
79. Diethyl N,N- <i>bis</i> (β -chloroethyl)amidophosphate	53h	bp ¹⁰	164–165.5°	53h	29
80. <i>bis</i> (β -Chloroethylthio)-N,N- <i>bis</i> (β -chloroethyl)-amidophosphate	48f	n_D^{25}	1.5525	48f	29
	...	d^{25}	1.472	48f	...
	...	bp ^{0.01}	155–160°	48f	...
81. Ethyl chloracetiminoester hydrochloride	11	mp	90–100°(dec.)	11	29, 41
82. N- β -Chloroethylaniline	11	bp ¹	80°	11	...
83. N,N'- <i>bis</i> (β -Chloroethyl)- <i>p</i> -phenylenediamine	42	mp	79°	42	29, 41
84. N,N'- <i>bis</i> (β -Chloroethyl)- <i>p</i> -phenylenediamine dihydrochloride	42	mp	212°(dec.)	42	29, 41
85. N,N'- <i>bis</i> (β -Chloroethyl)-N,N'-dinitroso- <i>p</i> -phenylenediamine	42	mp	106.5°	42	29, 41
86. N,N'- <i>bis</i> (β -Chloroethyl)-N,N'-dicarbethoxy- <i>p</i> -phenylenediamine	42	mp	102–103°	42	29, 41
87. 2-(β -Chloroethylamino)quinoline hydrochloride	48g	mp	149–152°	48g	29, 41
88. α -Benzylamino- β -chloro- β -phenylpropiophenone hydrochloride	47	mp	152–156°	58	29
89. β -Benzylamino- α -bromo- β -phenylpropiophenone hydrobromide	47	mp	147–149°	58	29
90. α -Benzylamino- β -bromo- β -phenylpropiophenone hydrobromide	47	mp	144–147°	58	...
<i>C. Derivatives of tertiary amines</i>					
91. N-Methylethyleneimine	...	...		...	29
92. β -Ethyleneiminopropionitrile	53j	bp ¹²	67–69°	53j	29
93. Methyl β -ethyleneiminopropionate	53j	bp ¹⁰	61–64°	53j	29
94. β -Chloroethyldimethylamine*	...	...		...	134
95. Dimethyl β -chloropropylamine	16	n_D^{21}	1.4214	16	29, 41
	...	d^{25}	0.899	16	...
	...	bp ³⁷	36.5–38°	16	...
96. Dimethyl β -chloropropylamine hydrochloride	16	mp	170–174°	16	...
97. Methyl <i>bis</i> (β -fluoroethyl)amine*	...	bp	123–124°	177e	177e
98. Methyl <i>bis</i> (β -chloroethyl)amine*	2, 5	n_D^{18}	1.4679	2	29, 41, 121, 134
	...	n_D^{25}	1.4682	115	...
	...	d^{25}	1.118	82	...
	...	d^{25}	1.1203	115	...
	...	bp ²⁰	50.0–50.5°	2	...
	...	vol ²⁰	2.487	15	...
99. Methyl <i>bis</i> (β -chloroethyl)amine hydrochloride*	2	mp	107–108°	2	29, 41, 129, 177a
100. Methyl <i>bis</i> (β -chloroethyl)amine formate	51a	...		...	...
101. Methyl <i>bis</i> (β -chloroethyl)amine picrate	51a	...		...	...
102. Boron fluoride complex of methyl- <i>bis</i> (β -chloroethyl)amine	50	...		...	...
103. Reaction product of methyl <i>bis</i> (β -chloroethyl)-amine and titanium tetrachloride	51c	...		...	...
104. Methyl <i>bis</i> (β -chloroethyl)amine oxide hydrochloride	...	...		...	29
105. Methyl <i>bis</i> (β -cyanoethyl)amine	53e	bp ¹⁰	177–187°	53e	29
106. Methyl <i>bis</i> (β -thiocyanoethyl)amine	23	Decomposition on distillation at 1.5 mm		23	29
107. Methyl <i>bis</i> (β -thiocyanoethyl)amine hydrochloride	23	mp	117–118°	23	29, 41
108. Methyl β -chloroethyl- β -hydroxyethylamine	12, 126	...		...	29, 41
109. Methyl β -chloroethyl- β -hydroxyethylamine hydrochloride	12	...		...	29
110. Methyl β -chloroethyl- β -hydroxyethylamine picrate	12	mp	72–73°	12	...
111. Methyl β -acetoxyethyl- β -chloroethylamine*	32, 126	n_D^{26}	1.4474	32	43, 106b
	...	bp ^{0.15}	54°	32	...
	...	bp ^{0.10}	46°	32	...
	...	bp ^{0.08}	41°	32	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity and vesicancy data
		Property			
112. Dimethyl β , β' -dichloro- <i>tert</i> -butylamine*	...	...		...	...
113. Methyl β -chloroethyl- β -chloropropylamine*	...	...		...	134
114. Methyl β -chloroethyl- β -chloropropylamine*	...	...		...	134
115. Diethyl β -chloroethylamine*	...	...		...	...
116. Diethyl β -chloroethylamine hydrochloride	16	mp	210–211.5°	16	...
117. Ethyl <i>bis</i> (β -chloroethyl)amine*	2, 10, 120	n_D^{26}	1.4639	2	29, 41, 134, 143
	...	d	1.083	2	...
	...	bp ^{1.5}	49.0–49.5°	2	...
	...	vol ²⁰	1.59	31	...
118. Ethyl <i>bis</i> (β -chloroethyl)amine hydrochloride*	2, 10	mp	139–140°	2	137
119. Ethyl β -chloroethyl- β -hydroxyethylamine picryl-sulfonate	17	mp	110–111°	17	29
120. β -Methoxyethyl <i>bis</i> (β -chloroethyl)amine	23	n_D^{26}	1.4671	23	29, 41
	...	d^{27}	1.108	23	...
121. β -Methoxyethyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	23	mp	133.5–134.5°	23	...
122. <i>tris</i> (β -Chloroethyl)amine	2, 5, 156	n_D^{20}	1.4962	156	29, 41, 118, 156
	...	bp ⁸	127°	156	...
	...	bp ¹⁵	141°	156	...
	...	mp	3.7°	156	...
	...	vol ²⁰	0.0779	31	...
123. <i>tris</i> (β -Chloroethyl)amine hydrochloride	2, 156	mp	130–131°	2	29, 41, 156
124. Boron fluoride complex of <i>tris</i> (β -chloroethyl)amine	50	...		...	...
125. <i>tris</i> (β -Chloroethyl)hydroxy ammonium chloride*	...	...		...	...
126. <i>tris</i> (β -Thiocyanoethyl)amine	...	...		...	106c
127. β -[<i>bis</i> (β -Chloroethyl)amino]propionitrile	23	n_D^{25}	1.4900	23	29, 41
	...	d^{20}	1.151	23	...
	...	bp ^{2.0}	126–130°	23	...
128. β -[<i>bis</i> (β -Chloroethyl)amino]propionitrile hydrochloride	23	mp	89.5–90.5°	23	...
129. N- β -Chloroethylmorpholine*	11, 23, 200	bp ²³	102–106°	11, 31	29, 134
	...	vol ²⁰	1.792	...	...
130. N- β -Chloroethylthiomorpholine*	11	bp ^{0.5}	77–80°	11	29
131. N- β -Chloroethylthiomorpholine hydrochloride*	11	mp	208–209°	11	...
132. N- β -Chloroethylthiomorpholine sulfone*	...	...		...	...
133. N- β -Chloroethylthiomorpholine sulfone hydrochloride*	...	...		...	...
134. Methyl- <i>bis</i> (γ -chloroallyl)amine*	...	...		...	...
135. Methyl- <i>bis</i> (β -chloropropyl)amine*	16, 130	n_D^{21}	1.5147	16	41, 134
	...	d^{25}	1.027	16	...
	...	bp ^{2.4}	55–57°	16	...
	...	vol ²⁰	1.898	31	...
136. Methyl- <i>bis</i> (β -chloropropyl)amine hydrochloride	16	...		...	...
137. 2-Propynyl- <i>bis</i> (β -chloroethyl)amine	32	n_D^{25}	1.4915	32	29
	...	d^{27}	1.139	32	...
	...	bp ¹	63–64°	32	...
138. 2-Propynyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	32	...		...	...
139. Allyl- <i>bis</i> (β -chloroethyl)amine*	2, 23	n_D^{29}	1.4739	2	29, 134, 148
	...	d	1.075	2	...
	...	bp ³	80°	148	...
	...	bp ²	62–63°	2	...
140. Allyl- <i>bis</i> (β -chloroethyl)amine hydrochloride*	2, 23	mp	91–92°	23	...
141. β -Chloroallyl- <i>bis</i> (β -chloroethyl)amine	32	bp ²	85–87°	32	29
142. β -Chloroallyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	32	mp	105–106.5°	32	...
143. Propyl <i>bis</i> (β -chloroethyl)amine*	16, 120	n_D^{27}	1.4629	16	29, 41, 134, 143
	...	d^{20}	1.092	16	148
	...	bp ¹	62–63°	16	...
	...	vol ²⁰	0.783	31	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties			Reference to toxicity and vesicancy data
		Property		Reference	
144. Propyl <i>bis</i> (β -chloroethyl)amine hydrochloride*	16, 120	mp	118–120°	16	137
145. β -Chloropropyl- <i>bis</i> (β -chloroethyl)amine*	...	...		...	...
146. Isopropyl- <i>bis</i> (β -chloroethyl)amine*	2, 9	n_D^{24}	1.4641	2	29, 41, 143
	...	d	1.053	2	...
	...	bp ^{2.5}	67.0–68.0°	2	...
	...	mp	13.7	83	...
	...	vol ²⁰	0.869	15	...
147. Isopropyl- <i>bis</i> (β -chloroethyl)amine hydrochloride*	2, 9	mp	210–213°(dec.)	2	41, 137
148. N- β -Chloroethylpiperidine	11	bp ²	40–41°	11	29, 41
149. N- β -Chloroethylpiperidine hydrochloride	11	mp	230°	11	...
150. Butyl- <i>bis</i> (β -chloroethyl)amine	16	n_D^{25}	1.4637	16	29, 41
	...	d^{26}	1.027	16	...
	...	bp ^{2.5}	89.0–89.5°	16	...
	...	vol ²⁰	0.321	31	...
151. Butyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	16	mp	96–97°	16	...
152. γ -Chlorobutyl- <i>bis</i> (β -chloroethyl)amine	...	...		...	29, 41
153. γ -Oxobutyl <i>bis</i> (β -chloroethyl)amine hydrobromide	...	...		...	29
154. <i>sec</i> -Butyl- <i>bis</i> (β -chloroethyl)amine	16	n_D^{25}	1.4655	16	29, 41
	...	d^{24}	1.028	16	...
	...	bp ¹	84–84.5°	16	...
	...	vol ²⁰	0.394	31	...
155. <i>sec</i> -Butyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	16	mp	132–138°	16	...
156. Isobutyl- <i>bis</i> (β -chloroethyl)amine	16	n_D^{27}	1.4597	16	29, 41
	...	d^{27}	1.0078	16	...
	...	bp ¹	81–81.3°	16	...
	...	vol ²⁰	0.508	31	...
157. Isobutyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	16	mp	107–108°	16	...
158. <i>tert</i> -Butyl- <i>bis</i> (β -chloroethyl)amine	16	n_D^{20}	1.4710	16	29, 41
	...	d^{21}	1.032	16	...
	...	bp ^{1.5}	68–69°	16	...
	...	vol ²⁰	0.581	31	...
159. <i>tert</i> -Butyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	16	mp	176.5–177.5°	16	...
160. β , β' , β'' -Trichloro- <i>tert</i> -butyl <i>bis</i> (β -chloroethyl)-amine	23	n_D^{25}	1.5226	23	29
	...	d^{26}	1.302	23	...
	...	bp ^{2.0}	138–140°	23	...
161. β , β' , β'' -Trichloro- <i>tert</i> -butyl <i>bis</i> (β -chloroethyl)-amine hydrochloride	23	mp	112.2–114.2°	23	41
162. β -Chloroethyl- <i>bis</i> (β -chloropropyl)amine*	...	...		...	...
163. β -Chloroethyl- <i>bis</i> (β -chloropropyl)amine hydrochloride*	...	...		...	...
164. Furfuryl <i>bis</i> (β -chloroethyl)amine	23	n_D^{25}	1.5033	23	41
	...	d^{25}	1.171	23	...
	...	bp ^{3.5–4.0}	106–107°	23	...
165. Furfuryl- <i>bis</i> (β -chloroethyl)amine hydrochloride	23	mp	88.5–89.5°	23	29, 41, 43
166. Tetrahydrofurfuryl- <i>bis</i> (β -chloroethyl)amine	23	n_D^{25}	1.4877	23	29, 43
	...	d^{29}	1.129	23	...
	...	bp ^{0.5}	82–84°	23	...
167. Tetrahydrofurfuryl- <i>bis</i> (β -chloroethyl)amine hydrochloride	23	mp	117–118°	23	...
168. <i>dl</i> -N-(β -Chloroethyl)-2-chloromethylpiperidine hydrochloride*	...	...		...	...
169. 6(or 7)-Chloroetronecane	30	n_D^{19}	1.4913	30	29
	...	bp ³⁰	111–112°	30	...
170. 6(or 7)-Chloro-1-chloromethyl-1,2-dehydropyrrolizidine hydrochloride	30	mp	122–123°	30	29
171. <i>tris</i> (β -Chloropropyl)amine	11	bp ³	99–103°	11	29
172. N-Ethyl-N-(β -chloroethyl)aniline	11	bp ^{0.1}	102–109°	11	29, 41
173. N,N- <i>bis</i> (β -Chloroethyl)aniline*	11	bp ^{0.7}	123°	11	29, 41
	...	mp	43–44°	11	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity and vesicancy data
		Property			
174. N,N- <i>bis</i> (β -Chloroethyl)- <i>p</i> -nitrosoaniline	53c	mp	72°	53c	29, 41
175. Cyclohexyl- <i>bis</i> (β -chloroethyl)amine	16	n_D^{25}	1.4940	16	29, 41
	...	d^{28}	1.077	16	...
	...	bp ^{1.4}	105–105.6°	16	...
	...	vol ²⁰	0.0383	31	...
176. Cyclohexyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	16	mp	174–175°	16	...
177. Benzyl- <i>bis</i> (β -chloroethyl)amine	16	n_D^{25}	1.5334	16	29, 41
	...	d^{25}	1.112	16	...
	...	bp ²	138–139°	16	...
178. Benzyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	16	mp	147.2–148.2°	16	41
179. Heptyl- <i>bis</i> (β -chloroethyl)amine	2	d	<1.0	2	29
180. Heptyl- <i>bis</i> (β -chloroethyl)amine hydrochloride	2	...		...	41
181. Phenethyl- <i>bis</i> (β -chloroethyl)amine	53g	bp ⁸	96°	53g	29
182. N, N, N', N'- <i>tetrakis</i> (β -Chloroethyl)ethylene-diamine dihydrochloride	49	...		...	29, 41
183. 1,3- <i>bis</i> (<i>bis</i> (β -Chloroethyl)amino) propane dihydrochloride	32	mp	138–139°	32	29
184. 1,3- <i>bis</i> [<i>bis</i> (β -Chloroethyl)amino]-2-chloropropane dihydrochloride	32	mp	141–141.2°	32	29
185. <i>bis</i> [β -(<i>bis</i> (β -Chloroethyl)amino)-ethyl] sulfide*	23	n_D^{25}	1.5287	23	29
	...	mp	24.2–24.7°	23	...
186. <i>bis</i> [β -(<i>bis</i> (β -Chloroethyl)amino)-ethyl] sulfide dihydrochloride	23	mp	137–138°	23	29, 41
187. N,N'- <i>bis</i> (β -Chloroethyl)-N,N'-dimethyl- <i>p</i> -phenylenediamine	42	mp	61°	42	29
188. 2-Chloromethylpyridine	53b	...		...	...
189. 2-Chloromethyl-5-methoxy- γ -pyridone hydrochloride	53d	mp	155°	53d	...
190. 2-(β -Chloroethyl)pyridine	11	bp ^{0.2}	57°	11	...
191. 2-(β -Chloroisopropyl) pyridine hydrochloride	53i	mp	122°	53i	...
192. N- β -Chloroethylcarbazole	59	mp	130–131°	59	29, 41
193. 3,6-Dibromo-N- β -chloroethylcarbazole	48a	...		...	...
194. N- β -Chloroethylacridan	48b	...		...	...
195. N- β -Chloroethylphenothiazine	48b	...		...	...
<i>D. Derivatives of quaternary ammonium salts</i>					
196. Trimethyl- β -fluoroethylammonium bromide*	...	mp	244° (dec.)	177d	177d
197. Dimethyl- <i>bis</i> (β -chloroethyl)ammonium chloride	...	...		...	29, 41
198. β -Acetoxyethyl- β -chloroethyldimethylammonium iodide*	126	mp	150° (dec.)	126	...
199. Methyl- <i>tris</i> (β -chloroethyl)ammonium chloride*	...	...		...	...
200. Methyl- <i>tris</i> (β -chloroethyl)ammonium sulfate*	...	...		...	...
201. Ethylvinyl- <i>bis</i> (β -chloroethyl)ammonium chloride	32	mp	192–196° (dec.)	32	29
202. Triethyl- β -fluoroethylammonium bromide*	...	mp	237° (dec.)	177c	177c
203. β -Carbamoxyethylethyl- <i>bis</i> (β -chloroethyl)ammonium chloride	23	...		...	29, 41
204. β -Fluoroethylpyridinium bromide*	...	mp	180°	177c	177c
205. N,N- <i>bis</i> (β -Chloroethyl)piperidinium chloride monohydrate	...	...		...	...
206. Polymer of methyl- <i>bis</i> (β -chloroethyl)amine ($n = 2$)	2	...		...	29
207. Polymer of methyl- <i>bis</i> (β -chloroethyl)amine	2	...		...	29
208. 1,4- <i>bis</i> (β -Chloroethyl)-1,4-diethylpiperazinium dichloride	...	...		...	29
209. Ammonium compound from 1 mole of methyl- <i>bis</i> (β -chloroethyl)amine and 2 moles of methyldiethanolamine	...	...		...	29

Detailed studies of the preparation of the four pilot plant, or manufacturing scale are reported in most important nitrogen mustards on a laboratory, the following references.

SECRET

Agent	References
HN1	2, 4, 10, 74, 87, 95, 97, 98, 99, 136, 182d
HN2	2, 4, 5, 82, 120, 158, 160, 182a, 185a, 185b, c, d, 192, 194, 196
HN3	2, 4, 5, 68, 80, 92, 96, 157, 170, 171, 172
Isopropyl- <i>bis</i> (β -chloroethyl)amine	2, 4, 83, 136

Work has been done on alternate syntheses which do not employ thionyl chloride. Results have been discouraging.^{2,4,46} The best alternate method for HN1 uses phosphorous trichloride in place of thionyl chloride, and gives yields approaching 75 per cent.⁴⁶ Other reagents used with less success are phosphorous pentachloride,²⁰⁵ phosgene,^{185b,d,193} and hydrochloric acid.^{185c}

The alkyl-*bis*(β -hydroxyethyl)amines and *tris*(β -hydroxyethyl)amine required for synthesis of the nitrogen mustards have been prepared commercially by reaction of primary amines or ammonia with ethylene oxide. Some work has been reported in the classified literature on such reactions and on the purification of technical ethanolamines for use as nitrogen mustard intermediates.^{98,159,160,161,165,167,168,169,172,185a,e,192,194,196}

Because of the possibility of a short supply of ethylene oxide in the event of large-scale nitrogen mustard manufacture, methods which did not employ ethylene oxide were investigated for preparing alkanolamines, particularly $\text{RN}(\text{CH}_2\text{CH}_2\text{OH})_2$ (where R is ethyl, methyl, or isopropyl). The most successful method developed utilizes formaldehyde, hydrogen cyanide, and ethyl alcohol as the basic raw materials and follows these steps:

- $\text{HCN} + \text{CH}_2\text{O} \longrightarrow \text{CH}_2\text{OHCN}$
- $\text{CH}_2\text{OHCN} + (\text{C}_2\text{H}_5\text{O})_2\text{CH}_2 \longrightarrow \text{C}_2\text{H}_5\text{OCH}_2\text{OCH}_2\text{CN} + \text{C}_2\text{H}_5\text{OH}$
- $2\text{C}_2\text{H}_5\text{OCH}_2\text{OCH}_2\text{CN} + 4\text{H}_2 \longrightarrow (\text{C}_2\text{H}_5\text{OCH}_2\text{OCH}_2\text{CH}_2)_2\text{NH} + \text{NH}_3$
- $2(\text{C}_2\text{H}_5\text{OCH}_2\text{OCH}_2\text{CH}_2)_2\text{NH} + (\text{C}_2\text{H}_5)_2\text{SO}_4 + \text{Na}_2\text{CO}_3 \longrightarrow 2(\text{C}_2\text{H}_5\text{OCH}_2\text{OCH}_2\text{CH}_2)_2\text{NC}_2\text{H}_5 + \text{Na}_2\text{SO}_4 + \text{H}_2\text{O} + \text{CO}_2$
- $(\text{C}_2\text{H}_5\text{OCH}_2\text{OCH}_2\text{CH}_2)_2\text{NC}_2\text{H}_5 + \text{HCl} + \text{C}_2\text{H}_5\text{OH} \longrightarrow (\text{CH}_2\text{OHCH}_2)_2\text{NC}_2\text{H}_5 \cdot \text{HCl} + 2\text{CH}_2(\text{OC}_2\text{H}_5)_2$

This "formal" route, in which formaldehyde cyanohydrin is converted to a less sensitive formal derivative before hydrogenation, is estimated to be capable of producing N-ethyl diethanolamine hydrochloride at a cost of 25 to 30 cents per pound, at an annual rate of 10,000,000 pounds.²⁰ A more direct

route, in which formaldehyde cyanohydrin is hydrogenated directly to diethanolamine (subsequently alkylated), gave poorer yields and appears to be a more expensive process.²⁰ Methyl-*bis*(β -hydroxyethyl)amine has been prepared successfully by hydrogenation of diethanolamine in the presence of formaldehyde.¹⁹ Other less advantageous routes to the alkanolamine intermediates for HN1, HN2, and HN3 have been explored.¹⁹ In addition to the standard method of preparation from isopropyl amine and ethylene oxide, isopropyl-*bis*(β -hydroxyethyl)amine has been prepared by hydrogenation of a mixture of acetone and diethanolamine,¹⁹ or by the reaction of ethylene oxide with isopropyl- β -hydroxyethylamine. The latter compound is prepared by hydrogenating a mixture of acetone and ethanolamine.¹⁹⁹

6.2.2

Physical Properties

The nitrogen mustards are oils of limited water solubility. They are miscible with ordinary organic solvents. Their physical properties have been extensively studied.^{2,4,6,7,15,25,26,31,52,80,82,83,87,115,118,156,164,167,168,169,183b,c} Some of the constants having most bearing on chemical warfare are presented in Table 2.

6.2.3

Chemical Properties

The nitrogen mustards are basic amines which form stable salts with strong acids such as hydrochloric acid. They are active alkylating agents, and the physiological reactions responsible for their toxicity are primarily alkylations. Their reactions from the biochemical, physicochemical, and physiological mechanism standpoints have been studied in great detail and are summarized in Chapters 19, 20, and 21. A primary intermediate in their reactions is a 1-(β -chloroethyl)ethylenimonium ion, formulated below for HN2, which is analogous with the ethylene-sulfonium compound^b intermediate in the reactions of H (see Chapters 19 and 20).^{3,4,8,13,14,17,18,24,26,33,122,125,127,130,135,151,181a,b}

Self-alkylation is responsible for the dimerization which occurs slowly when the lower molecular weight alkyl-*bis*(β -chloroethyl)amines are allowed to stand. The reaction is rapid in the presence of water. It results in the deposition of crystalline solids such as the "dichlorocyclic dimer" which is formed from HN2:

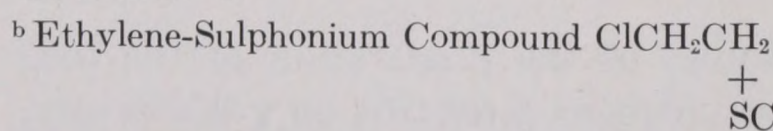


TABLE 2. Physical properties of HN1, HN2, HN3, and isopropyl-*bis*(β -chloroethyl)amine in comparison with those of H.

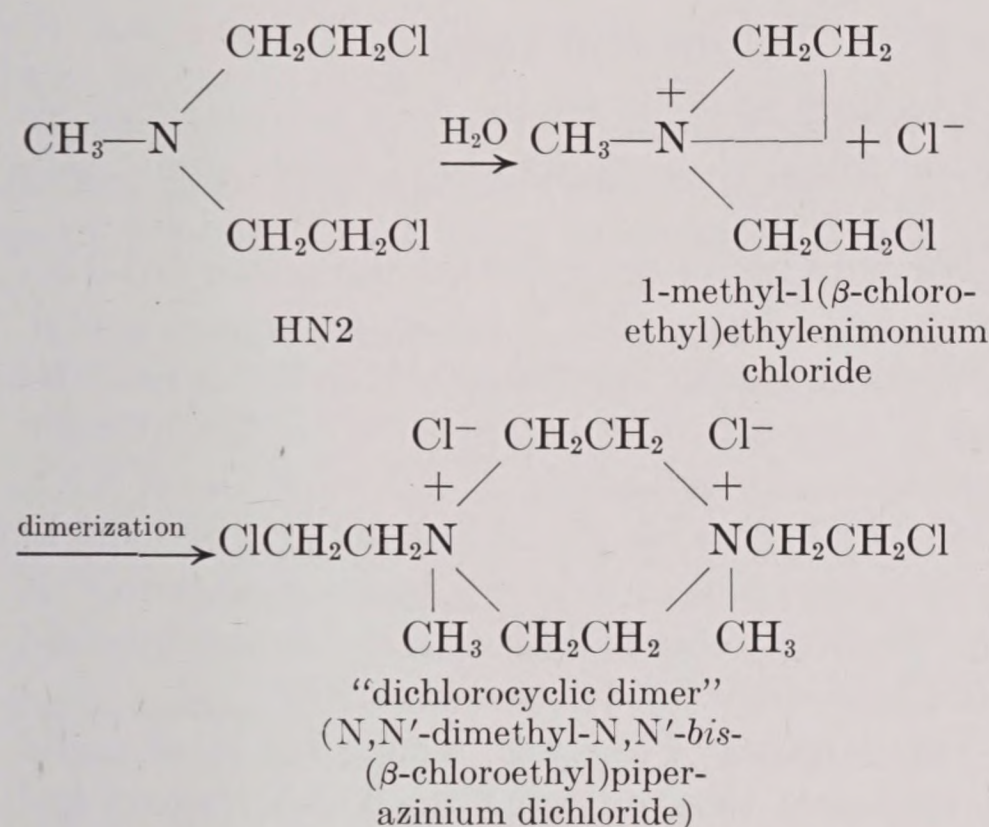
Property	Agent				
	H	HN1	HN2	HN3	Isopropyl- <i>bis</i> (β -chloroethyl)amine
Molecular weight	159	170	157	204.6	184
Freezing point (C)	14.2 ³¹	-33 $\pm$ 87,88	-70 $\pm$ 82,91,115	-3 $\pm$ 15,80,188	13.7 ⁸³
Density (g/ml at 25 C)	1.27 ⁶⁷	1.09 ⁸⁷	1.12 ⁸²	1.23 ^{15,80,188}	1.053 ²
Vapor pressure (mm Hg)					
at 10 C	0.0319*, ³¹	0.0773 ³¹	0.130 ^{†,‡}	0.00272 ^{§,31}	0.0364 ^{6,15}
at 25 C	0.112	0.250 [†]	0.427	0.0109	0.130
at 40 C	0.346	0.722	1.25	0.0382	0.410
Volatility (mg/l)					
at 10 C	0.288*, ³¹	0.744 ³¹	1.15 ^{†,‡}	0.0315 ^{§,31}	0.380 ^{6,15}
at 25 C	0.96	2.29	2.58	0.120	1.29
at 40 C	2.83	6.29	10.0	0.400	3.87

* The values are in good agreement with those reported in reference 183a.

† Compare 0.239 mm Hg at 25C.⁸⁷

‡ Compare reference 183b.

§ Compare 0.0656 mg/l at 25C,¹⁸⁸ 0.011 mm Hg at 25C,^{183c} 0.014 mm Hg at 25C,¹⁶⁴ and 0.112 mg/l at 25C.⁶ The values reported in reference 15 are now believed to be too high.



the rate is so slow if the agent is dry that its storage stability can be considered so good as to offer no practical limitations on its use as a chemical warfare agent.^{100,112,118,173}

HN1 is also considered to be sufficiently stable for storage but the margin of safety is much less than in the case of HN3 and the use of stabilizers is necessary or desirable. The amine should be dry for maximal stability; addition of 10 per cent kerosene² plus 1 per cent hexamethylenetetramine²⁷ as stabilizers has been recommended.

HN2 was originally considered sufficiently stable for use in temperate climates by the British,¹⁴⁵ but the low stability of production lots of this agent and the occurrence of an explosion during storage in one instance have led to a revision of this point of view. In one investigation HN2 was found to be only one-sixth as stable as HN1.⁴

Isopropyl-*bis*(β -chloroethyl)amine was found to be 25 times as stable as HN1.⁴

HN1 and HN3 can be satisfactorily thickened with various polymeric materials.⁹⁴

STABILITY ON EXPLOSION IN MUNITIONS

The explosion stabilities of HN1, HN2, and HN3 appear to fall in the same order as their stabilities upon storage. All available evidence indicates that HN3 is exceedingly stable, certainly not inferior to H in this regard. HN3-filled 105-mm shell, 75-mm shell, HE/Chem. 6-inch howitzer shell, and M47A2 bombs have been exploded without qualitative evidence of decomposition of the charging.^{65,79,86,107} The plan of the Germans to use HN3 in high explosive-chemical shell offers confirmatory evidence.

6.2.4

Stability

STORAGE STABILITY

The alkyl-*bis*(β -chloroethyl)amines as a class dimerize on storage and deposit crystalline dimers at rates which decrease with increasing size and branching of the alkyl group. The stabilities of many of the nitrogen mustards prepared for toxicological examination have been measured by heating them in closed systems at 60 C and determining the rate of formation of solid dimer.^{2,5,10,16,23} The rate of dimerization is not appreciably accelerated in the presence of steel, brass, or solder.^{1,5,27} In the cases of HN1 and HN3 pressure development is not a problem.^{1,27,173}

HN3 is the most stable of the more important nitrogen mustards. It slowly deposits a crystalline dimer on long standing at elevated temperatures, but

HN1 is certainly less stable than HN3, although not necessarily so much more so as to negate its practical use as a chemical warfare agent. HN1-filled 75-mm shell, 4.2 chemical mortar shell, and M70 bombs have been exploded without evidence of gross decomposition except in 75-mm shell under conditions more severe than are encountered in the field.^{64,86,89} However, in the most quantitative investigation that has been made, one of six M47A2 bombs charged HN1 flashed on static detonation.⁶⁶ Although considerable toxicologically effective vapor was subsequently evolved from the terrain contaminated by the explosions in these tests, the dosages were less by an amount approaching 30 per cent than would have been predicted from similar tests with H had no decomposition of HN1 occurred during the explosion or, subsequently, on the contaminated terrain.⁶⁶

The available data for HN2 are of a rather qualitative nature. It appears that this agent can be dispersed without complete destruction by explosion of various chemical munitions but that the toxicological effects of the initial clouds so produced are inferior to those produced by H or HT.^{79,107,115,147,189,190}

STABILITY ON TERRAIN

Two important characteristics determining rate of inactivation on soil and vegetation are solubility in water and rate of reaction with water. The approximate data given in Table 3 lead to the prediction of

TABLE 3. Characteristics influencing rate of inactivation of H, HN1, HN2, and HN3 on terrain.

Agent	Approximate solubility in water at room temperature (ppm)	Approximate half-life in water at 25 C (minutes)
H	500	8
HN1	4,000 ±	1.3
HN2	13,000 ±	4.0
HN3	80	2.4

greater losses with HN1 and HN2 than with HN3 and H. This prediction is confirmed by the available field data. There is no evidence in the semiquantitative data of bomb and annulus trials that losses on moist terrain are greater for HN3 than for H, in spite of the greater persistence of HN3.⁶⁵ The results of British annulus trials with HN1 and HN2 on alkaline sod (Porton downland) indicate that ground losses are significantly greater than in the case of H.^{115,155} Although the initial dosages of evolved

HN1 and HN2 vapor were greater than those of H, as predicted from the relative volatilities, the total dosage (*Ct*) of evolved vapor was in the cases of HN1 and HN2 only one-half of that anticipated from similar trials with H. In annulus trials with HN1 and H in Florida, large drops were used on relatively dry terrain and the 4-hour vapor dosages failed to reveal significantly greater ground losses for HN3 than for H.⁶⁶ As stated above, however, in trials with single, statically fired bombs the vapor evolution of HN1 was somewhat less than would have been predicted on the basis of tests with H. Part of the loss may have occurred during the explosion of the bombs, and part subsequently on the terrain. Terrain losses would have been facilitated by the dispersion of the agent into small droplets during the explosion.

6.2.5 Detection and Analysis

Excellent methods for the detection and analysis of the nitrogen mustards are available (see Chapters 34 and 37).

For purposes of detection the use of the DB-3 reagent (see Chapter 34) is perhaps the most useful. As used in the United States M9 Detector Kit, this test is approximately as sensitive for the nitrogen mustards as for H. Collection of 0.1 to 0.2 μg of HN1, HN3, or H from air containing 0.2 $\mu\text{g}/\text{l}$ or more of these agents suffices to give a positive reaction.¹¹² A supplementary test to differentiate nitrogen mustard from H is somewhat less sensitive.

For purposes of analysis, among the most useful methods are those utilizing the DB-3 reagent and those dependent upon the mercurimetric titration of the chloride obtained by hydrolysis from the nitrogen mustard (see Chapter 37).

6.2.6 Decontamination and Protection

The gas mask canister offers complete protection against the nitrogen mustards.

In general, standard methods of decontamination effective for H are useful for the nitrogen mustards, but destruction of the nitrogen mustards by chemical reaction with bleach or with currently used chloramides is notably less efficient and rapid than in the case of H¹¹⁷ (see Chapters 24 and 32).

6.3 CHEMICAL STRUCTURE IN RELATION TO TOXICOLOGICAL POTENCY

Among the nitrogen mustards and related compounds that have been studied (see Table 1), the

highest toxicological potency is found among the tertiary *bis*(β -chloroethyl)amines, and, in particular, in HN1, HN2, HN3, and the propyl and isopropyl analogs of HN1. The toxicity, vesicancy, and eye-injuring action of these compounds is reviewed in the following sections.

6.4 TOXICOLOGY

6.4.1 Detectability by Odor and Sensory Irritation

HN1, HN2, and HN3 are markedly less detectable by odor or sensory irritation than is H. Testimony to the insidiousness of the vapors of all three nitrogen mustards comes from plant accidents in which men, informed of the potential hazard, were incapacitated without being aware of having been exposed until eye and respiratory symptoms developed after a lapse of several hours.^{69,174,175}

Laboratory (osmoscopic) determinations of the median detectable concentrations of H, HN1, HN2, and HN3 are given in Table 4. Attention is directed

TABLE 4. Median detectable concentrations of H, HN1, HN2, and HN3 as determined in the laboratory by the osmoscopic technique.

Agent	Purity	Median detectable conc. ($\mu\text{g/l}$)	Reference
H	Plant run Levinstein	0.6	90
	Vacuum-distilled	1.2	102
	Thiodiglycol	1.8	102
HN1	Plant run	13	90
	Pure	17	85
HN2	Pure	38	76
HN3	Plant run	15 $\pm$	104a
	Pure	?	

only to the relative values because the absolute magnitudes of the concentrations are not necessarily of significance for field conditions.^c Median detectable concentrations as determined in a man-chamber were for H in the order of 1 $\mu\text{g/l}$ ¹⁸⁶ or even less;¹⁵⁶ for HN2, 13 $\mu\text{g/l}$;¹¹⁵ and for HN3, 8.5 $\mu\text{g/l}$.¹⁴⁶

HN1 and HN2 possess faint "fishy or soapy"

^c Use of a technique in which observers in the field walked upwind toward a source of HN1 vapor until they detected an odor and then took a sample of air for analysis revealed that this agent could be detected at an *average* concentration in air of 0.02 $\mu\text{g/l}$, although the characteristic fishy odor was apparent only at much higher concentrations.¹⁵⁵ Presumably use of this technique with H and other nitrogen mustards would give values correspondingly low in relation to the median detectable concentrations as determined in the laboratory.

odors. The pilot plant HN3 that was made in England has a faint geranium-like odor. Inasmuch as oral reports indicate that laboratory-prepared samples do not possess this odor, it may be suspected that the geranium-like odor was due to an impurity, possibly associated with the preparation of the material in equipment previously used for lewisite. This pilot plant material was used in the osmoscopic determination cited in the preceding paragraph. Thus, it is possible that other samples of plant run HN3 would be even more odorless, and therefore more insidious.

6.4.2 Toxicity

Toxicity data for animals totally exposed to airborne HN1, HN2, and HN3 are set forth in Tables 6, 7, and 8. From the summary presented in Table 5 it

TABLE 5. Summary of toxicities of H, HN1, HN2, and HN3 in the form of vapors.

(See Tables 6, 7, and 8 of this chapter and Table 5 of Chapter 5 for more detailed data.)

Agent	$L(Ct)_{50}$ (mg min/m ³)		Estimated relative toxicity (H = 100)
	Mouse* (t = 10 min)	Range for other species†	
H	1,100	600–2,800	100
HN1	900	500–3,000	$\approx$ 100
HN2	2,600	1,000–6,000	50
HN3	550	500–2,000	$\geq$ 100

* Fifteen-day observation period.

† Approximate.

appears that, considering all species, HN3 is somewhat more toxic than H, HN1 about as toxic as H, and HN2 about one-half as toxic as H.

The nominal LC_{50} 's of propyl-*bis*(β -chloroethyl)amine and isopropyl-*bis*(β -chloroethyl)amine for mice exposed 10 minutes and observed 10 to 12 days are 0.16 to 0.17 mg/l.^{29,81} These figures, as well as less complete data for larger species,^{44a,143} indicate that the propylamines possess toxicities intermediate between those of HN1 and HN2. References to toxicity data for other nitrogen mustards and related compounds are given in Table 1.

The $L(Ct)_{50}$'s of HN1, HN2, and HN3 appear to be relatively independent of exposure time over the range from a few minutes to several hours (see Tables 6, 7, and 8).

Mortality data for animals exposed to the nitrogen mustards often exhibit great variability.^{37,44k,m,n,131,139,140,143} Among the factors which may contribute to this variation are presence of aerosol, wind speed or

TABLE 6. Toxicity of HN1. The animals were totally exposed. $L(Ct)_{50}$'s that are estimated very approximately are given in parentheses.

Species	$L(Ct)_{50}$ (mg min/m ³)	Exposure time (min)	Observation period (days)	Analytical (A) or nominal (N) conc.	Number of animals	Notes	Reference
Mouse	900	10	15	A	280	Low-flow chamber	37
	900	10	15	A	89	High-flow chamber	37
	1,300	10	10	N	140	Low-flow chamber	85
	<1,200	30	15	A	30	Static chamber	143
	960	20-100	15	A	140	Large chamber; 90 F; wind	
	1,100	20-100	10	A	140	speed without effect	104e
Rat	(750)	10	30	N	10	Low-flow chamber	37, 44a
	<1,200	30	15	A	34	Static chamber	143
	860	20-100	10 and 15	A	84	Large chamber; 90 F; wind	104e
						speed without effect	
Guinea pig	(2,500)	10	30	N	18	Low-flow chamber	37, 44a
	(1,500-3,000)	30	15	A	36	Static chamber	143
Rabbit	(1,000-3,000)	10	30	N	5	Low-flow chamber	37, 44a
	900	30	15	A	66	Low-flow chamber; 90 F	71
	(1,000)	30	15	A	18	Low-flow chamber; 73 $\pm$ F	71
	(>4,000)	30	15	A	15	Static chamber	143
	900	20-100	15	A	84	Large chamber; 90 F; wind	104e
	1,100	20-100	10	A	84	speed without effect	
	910	360	15	A	54	Low-flow chamber; 90 F	71
Cat	(400)	10	10-30	N	12	Low-flow chamber	37, 44a
Dog	(800)	10	10-30	N	14	Low-flow chamber	37, 44a
Goat	(1,500-3,000)	30	15	A	9	Static chamber	143
Monkey	(1,500)	10	15	N	6	Low-flow chamber	37

flow rate, temperature, use of an anesthetic, and variable delayed deaths due to secondary infections.

Wind speed or flow rate is only of marked importance for toxicity when aerosol is present.^{37,104b} HN3 when present in part as fine drops is much more toxic at high flow rates than at low flow rates.^{37,140} At high flows a greater liquid dose is deposited on the skin, from which it may be absorbed after exposure both directly and indirectly as a result of licking and inhalation of vapor.^{37,140}

A number of data are available on the toxicities of the nitrogen mustards to animals exposed totally, by inhalation only, and by body only.^{37,131,144} In the case of mice totally exposed to HN3, the absorption of the agent from the body surface compares in importance with that directly inhaled.³⁷ It is doubtful, however, that casualties among troops in the field would be produced by the systemic effects of nitrogen mustard absorbed through the skin except when the exposures are already more than sufficient to produce vesicant effects of incapacitating severity.^{104,111}

Relatively high concentrations of the nitrogen mustards produce symptoms of irritation during exposure^{64,71,77,78,81,85,121,131} but lower concentrations

are without immediate effect.^{71,131} Symptoms then develop only after a latency of one to several hours and the most conspicuous pathological changes appear in the eyes and respiratory tract.^{64,71,93,121}

Death in lethally dosed animals is usually delayed for one day to two or more weeks, depending on dosage. Detailed pathological studies have been made.^{77,78,81,85,121,139,140,141,143}

Data on the toxicities and pathological actions of nitrogen mustards administered percutaneously, orally, and by injection are presented in Chapter 22. From the practical point of view it may be noted that production of casualties from the drinking of contaminated water could easily occur. The available chemical tests are, however, sufficiently sensitive to reveal potentially dangerous concentrations of the nitrogen mustards and their toxic products of partial hydrolysis^{25,104c} (see also Chapter 34).

6.4.3 Vesicant Action^d

The nitrogen mustards both as liquids and as vapors are potent vesicants. They are effective both on bare skin and through clothing. Vesicant action

^d No attempt is here made to duplicate material presented in Chapter 23.

TABLE 7. Toxicity of HN2. The animals were totally exposed. $L(Ct)_{50}$'s that are estimated very approximately are given in parentheses.

Species	$L(Ct)_{50}$ (mg min/m ³)	Exposure time (min)	Observation period (days)	Analytical (A) or nominal (N) conc.	Number of animals	Notes	Reference
Mouse	5,100	2	10	N	200	Low-flow chamber	77
	(6,000)	2	25 ±	A	40	Static chamber	121
	(2,000-6,000)	5	25 ±	A	40	Static chamber	121
	2,600	10	15	A	138	Low-flow chamber	29, 44b
	5,600	10	10	N	240	Low-flow chamber	77
	(2,000-7,000)	10	10	N	40	Static chamber	177b
	(2,000-6,000)	10	25 ±	A	30	Static chamber	121
	(2,000)	20	25 ±	A	40	Static chamber	121
	5,700	30	10	N	160	Low-flow chamber	77
	(1,500)	30	25 ±	A	50	Static chamber	121
	(3,000-4,000)	60-120	?	A	36	Low-flow chamber	131
	(4,000-5,000)	240-450	?	A	54	Low-flow chamber	131
	(600-1,200)	2	18	A	24	Static chamber	121
	(1,500)	5	<10	A	40	Static chamber	121
Rat	<4,400	10	15	N	12	Low-flow chamber	44a
	(≤2,000)	10	<10	A	30	Static chamber	121
	(1,750)	10	10(?)	N	24	Static chamber	177b
	(<1,800)	20	<10	A	30	Static chamber	121
	(1,000-3,000)	30	<10	A	30	Static chamber	121
	(2,000-3,000)	60-120	?	A	26	Low-flow chamber	131
	(2,000)	120-360	14	A	56	Low-flow chamber	131
	(2,000-4,000)	240-450	?	A	38	Low-flow chamber	131
	(2,000-3,000)	240-510	14	A	40	Low-flow chamber	131
	(>1,200)	2	?	A	24	Static chamber	121
	(3,000)	5	19	A	24	Static chamber	121
	(5,500)	10	15	N	12	Low-flow chamber	44a
	(3,500-7,000)	10	10(?)	N	16	Static chamber	177b
	(3,000-6,000)	10	5	A	20	Static chamber	121
Guinea pig	(4,000-8,000)	20	19	A	30	Static chamber	121
	(3,000-6,000)	30	7	A	30	Static chamber	121
	(>3,800)	60-120	?	A	8	Low-flow chamber	131
	(2,500-5,000)	240-450	?	A	14	Low-flow chamber	131
	(>1,200)	2	25	A	24	Static chamber	121
	(1,000-3,500)	5	26	A	24	Static chamber	121
	(4,400)	10	15	N	4	Low-flow chamber	44a
	(7,000-14,000)	10	10(?)	N	12	Static chamber	177b
	(3,000)	10	15	A	19	Static chamber	121
	(2,000-8,000)	20	28	A	30	Static chamber	121
	(3,000-6,000)	30	26	A	29	Static chamber	121
	<1,400	10	10-30	N	8	Low-flow chamber	44a
	(2,000)	10	10-30	N	4	Low-flow chamber	44a
	(1,000)	2	13	A	8	Static chamber	121
Cat	(<800)	5	22	A	8	Static chamber	121
	(<1,700)	10	?	A	6	Static chamber	121
	(<2,000)	20	?	A	8	Static chamber	121
	(1,000-2,000)	30	?	A	10	Static chamber	121
Dog							
Goat							

through clothing is of more practical importance than action on bare skin because usually most of the body surface, including the areas that are at the same time the most sensitive and the most critical for incapacitation, is clothed. In addition to vesicancy through unimpregnated clothing, the degree of protection offered by chloramide-impregnated

clothing and clothing containing activated carbon requires consideration.

A further differentiation must be made between situations in which the skin is relatively cool and dry and situations in which it is hot and moist. As in the case of H, high temperatures and humidities and physical exercise augment the sensitivity of men to

TABLE 8. Toxicity of HN3. The animals were totally exposed. $L(Ct)_{50}$'s that are estimated very approximately are given in parentheses.

Species	$L(Ct)_{50}$ (mg min/m ³)	Exposure time (min)	Observation period (days)	Analytical (A) or nominal (N) conc.	Number of animals	Notes	Reference
Mouse	(1,700)	<2	18	A	132	Fine aerosol	144
	500-600	10	14-15	A	58	Aerosol-free vapor	104a
	590	10	15	A	230	Low-flow chamber	37
	300	10	15	A	60	High-flow chamber; aerosol present	37
	(165)	10	15	A	20	Vapor; wind tunnel; 95 F	37
	1,700	10	10	N	160	Low-flow chamber	78
	570	10-100	15	A	139	Vapor, 90 F, 85% humidity	104c
Rat	(800)	0.25-2	20	A	104	Fine aerosol	144
	1,700	10	15	N	28	Low-flow chamber	54
	(800-1,500)	10	15	A	18	Low-flow chamber	37
	($\geq 1,000$)	30	?	A	50	Static chamber; 85 F	139
	670	10-100	15	A	69	Vapor, 90 F, 85% humidity	104c
	>2,300	10	?	N	10	Low-flow chamber	37
Guinea pig	>1,000	30	?	A	45	Static chamber; 85 F	139
Rabbit	(585)	3-15	15	A	12	Vapor; wind tunnel; 5.5 mph; 95 F	37
	(1,000-3,000)	10	10	N	11	Low-flow chamber	37
	(500)	10-18	15	A	8	Low-flow chamber; vapor only; 100 F	37
	(830)	18-50	15	A	30	Low-flow chamber; vapor only; 72 F	37
	(>1,000)	30	?	A	31	Static chamber; 85 F	139
	635	10-100	15	A	70	Vapor; 90 F, 85% humidity	104c
	550 $\pm$	long	15	A	60 $\pm$	Field tests	65
Cat	(400-1,000)	10	?	A	32	Low-flow chamber	37
Dog	(400-1,500)	10	?	A	36	Low-flow chamber	37
	<1,350	30	?	?	?	Low-flow chamber	187
Goat	(500-1,000)	30	?	A	18	Static chamber; 85 F	139

the vesicant effects of the nitrogen mustards (see Chapter 23).

So far as is known the time course of development of injury and incapacitation due to skin injury appears to be comparable for H and the nitrogen mustards (see Chapters 5 and 23). Some evidence exists that nitrogen mustard burns are shallower than H burns and heal more quickly.^{69,108,117,156} On the other hand nitrogen mustard burns have been referred to as more tender and painful than H burns.^{69,191} However, a sufficiently complete and realistic determination by means of performance tests of the relative extent and duration of incapacitation produced by lesions due to H, HN1, HN2, and HN3 remains to be made. Thus at present evaluations must be based principally on lesion-producing effectiveness rather than on the more pertinent criterion of casualty-producing effectiveness. Furthermore there is no information as to the effects of large dosages of nitrogen mustard vapors upon masked troops. In the case of H it is known that severe exposures under tropical

conditions produce incapacitation within one hour of exposure due to temporarily incapacitating nausea and vomiting followed rapidly by the development of very severe cutaneous injury.¹¹³ No evidences of systemic injury have been apparent in any of the man-chamber trials with HN1 and HN3 at the relatively low dosages that have been utilized.^{104b,c,e,111}

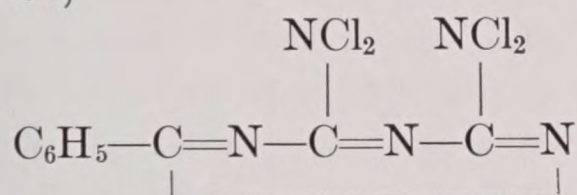
VESICANCY OF THE LIQUIDS

1. *Cool and temperate conditions.* For the production of lesions on bare skin when free evaporation is permitted and decontamination is not practiced, the order of vesicant potency is $H > HN3 > HN2 > HN1$. The relative weights of the small liquid drops required to produce blisters at 50 per cent of the sites of application are:^{39,44c,e,f,h,i,j,91,108}

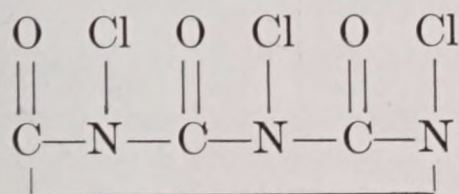
H	1
HN3	2-4
HN2	4-8
HN1	>8

The agents fall in the same relative order when evaluated by more realistic tests in which the sizes of the lesions produced by large drops are compared.^{148,154,191} None of the other nitrogen mustards and related compounds are as vesicant as HN2 or, probably, as vesicant as HN1 (see Table 1).^{41,148,191}

When *effective* decontamination is practiced 1 to 5 minutes after contamination, H produces markedly greater lesions than HN3.¹⁹⁷ The positions of HN1 and HN2 are not known with certainty; HN2 may be only slightly inferior to H¹⁹¹ and HN1 somewhat inferior to HN2.¹⁴⁹ Antivesicant ointments (i.e., United States M5 and British A.G. No. 6) available to Allied troops during World War II do not destroy the nitrogen mustards as they do H, but their bases are good solvents for the nitrogen mustards. The latter are effectively decontaminated by solvent and mechanical action when large amounts of ointment are applied and then wiped off.^{104a,118,197} If the ointment is left in place on the skin, the dissolved but undestroyed nitrogen mustard may slowly exert its vesicant action and the lesions produced by small doses of H and HN3 become comparable in severity.^{34,44f,g} Dilute acids also exert a solvent action on the nitrogen mustards, and such oxidizing agents as permanganate in aqueous solution may be utilized as decontaminants. In addition, some chloramides not in general use by the Allies during World War II do destroy nitrogen mustard readily. Notable among these are S-436,



and the German Decontaminant 40,



(see Chapter 24).

Through one or two layers of unimpregnated cloth the order of lesion-producing potency is $\text{H} > \text{HN2} > \text{HN1} \approx \text{HN3}$.^{91,148,149} The order found for bare skin is modified because of the importance of vapor pressure for the transport of the agent through the cloth and to the underlying skin.

Through cloth impregnated with CC-2 the nitrogen mustards gain in effectiveness relative to H because of the comparative ineffectiveness of CC-2 as a decontaminant for nitrogen mustard. Laboratory

data insufficient to permit a conclusive estimate suggest the following order of potency: $\text{HN2} > \text{H} > \text{HN1} > \text{HN3}$.⁹¹ Realistic trials under field conditions are lacking.

2. *Hot and humid conditions.* The scanty available data do not permit an evaluation of the relative potencies of the three liquids under tropical conditions. The lesions produced by small doses of the liquids on resting men and on men exercised to the point of sweating under temperate conditions suggest that the differences which would be observed among the agents if they were tested under severe tropical conditions might be less pronounced than those which have been obtained on relatively cool, dry skin.^{44j}

VESICANCY OF THE VAPORS

1. *Laboratory evaluation of potency.* Laboratory data which relate to the production of lesions on limited areas of the skin of the forearms of men not acclimated to hot summer weather and exposed under relatively moderate ambient conditions of temperature and humidity demonstrate that on a dosage basis HN3 is equal to or slightly more effective than H, that HN2 is definitely inferior to both H and HN3, and that HN1 is greatly inferior to each of the three other agents (Tables 9 and 10).^{28,36}

TABLE 9. Vapor train tests of the vesicant potencies of the vapors of H, HN1, HN2, and HN3.²⁸

The subjects were at rest. $T = 80 \text{ F}$.

Agent	Analytical Ct (mg min/m ³) for 50 per cent responses		Relative dosage*
	Erythemas	Blisters	
H	<430	2,500	1
HN1	2,700 ±	>21,000	>8
HN2	1,200 ±	5,800	2+
HN3	400 ±	1,800	0.7 ±

* Reciprocal of vesicant potency.

TABLE 10. Vapor cup tests of the vesicant potencies of the vapors of H, HN1, and HN3.³⁶

The subjects were at rest. $T = 72-73 \text{ F}$.

Agent	Estimated median vesicating dosage in mg min/m ³ ($t = 5-60 \text{ min}$)		Relative dosage*
H	3,500		1
HN1	18,000		5+
HN3	3,700		1.1

* Reciprocal of vesicant potency.

These relationships for H, HN1, and HN3 are confirmed by arm-chamber studies at high temperatures

TABLE 11. Basic man-chamber tests with H and nitrogen mustard vapors: United States Army data.^{104b,c,d,e}
T = 90 F. Relative humidity = 85 per cent. All subjects wore gas masks, shoes, and socks.

Season	Genital protection	Additional clothing and protection	Number of men	Vapor dosage (mg min/m ³)	Exposure time (min)	Effects
<i>H</i>						
Summer	CC-2 impregnated shorts	None	3	106	10	Moderate erythema of neck, back, and legs.
Summer	CC-2 impregnated shorts	None	6	200	20 ±	Severe erythema of neck, thorax, abdomen, and legs; some delayed superficial vesication.
<i>HN1</i>						
Summer	CC-2 impregnated shorts	None	3	107	11	No effects.
Summer	CC-2 impregnated shorts	None	3	211	22	No effects.
Summer	CC-2 impregnated shorts	None	3	285	30	Questionable erythema of neck.
Summer	CC-2 impregnated shorts	None	3	520	34	Mild erythema of neck; $\frac{1}{3}$ mild erythema of back.
Summer	CC-2 impregnated shorts	None	3	689	41	Mild erythema of neck and body.
Summer	CC-2 impregnated shorts	None	3	940	44	$\frac{1}{3}$ moderate and $\frac{2}{3}$ moderate erythema of upper trunk.
Summer	CC-2 impregnated shorts	None	3	1,030	29	$\frac{2}{3}$ moderate erythema of axillary folds; $\frac{2}{3}$ mild erythema of upper back and neck.
<i>HN3*</i>						
Winter or early spring	Unimpregnated shorts	None	2	90	15	No genital injuries: minimal erythema over exposed skin, marked over neck, back, and anterior axillary folds.
Winter or early spring	Carbon-containing shorts	None	2	90	15	
Winter or early spring	Carbon-containing shorts	None	3	150	25	
Winter or early spring	Carbon-containing shorts	None	4	200	?	$\frac{3}{4}$ slight and $\frac{1}{4}$ moderate erythema of trunk and neck; $\frac{3}{4}$ minimal erythema on legs.
Winter or early spring	Carbon-containing shorts	None	3	250	?	Slight erythema of trunk, moderate erythema of neck; $\frac{2}{3}$ minimal erythema of legs.

* Wind speed in the chamber seemed to be without effect and has been disregarded in compiling this table.

TABLE 11 (Continued).

Season	Genital protection	Additional clothing and protection	Number of men	Vapor dosage (mg min/m ³)	Exposure time (min)	Effects
Winter or early spring	Carbon-containing shorts	None	HN3* 8	300	?	$\frac{2}{8}$ slight, $\frac{3}{8}$ moderate, and $\frac{3}{8}$ marked erythema of trunk; more pronounced erythema of neck; minimal erythema of legs.
Winter or early spring	Carbon-containing shorts	None	4	350	?	Areas of vesication on trunk and neck; marked erythema with edema and moist desquamation of ears and preauricular areas; slight erythema of scalp; minimal erythema of legs.
Winter or early spring	Carbon-containing shorts	Nonimpregnated 2-piece herringbone twill suit, M5 ointment on neck.	4	350	?	$\frac{2}{4}$ slight erythema of neck; $\frac{2}{4}$ vesication of neck.

* Wind speed in the chamber seemed to be without effect and has been disregarded in compiling this table.

and humidities with the exception that, when tests were made with sweating observers acclimated to hot summer weather, HN1 assumed a much more favorable relative position, requiring only 1.2 to 1.6 times the dosage of H to produce equivalent lesions.¹⁰⁹ The effectiveness of H, HN1, and HN3 vapors in these tests was little affected by the interposition of a layer of unimpregnated cloth.¹⁰⁹

2. *Man-chamber evaluation of potency.* The only available man-chamber tests of the effects of nitrogen mustard vapors on observers wearing no clothing or unimpregnated clothing are summarized together with representative data for H in Tables 11 and 12.^{104b,c,d,e,110,111} These data relate to a chamber temperature of 90 F and relative humidities of 65 and 85 per cent, and to the production of injuries corresponding only to relatively mild partial disability.¹¹³

Although the two groups of data show some discrepancies, it seems reasonable to conclude that, in warm or hot weather and against troops provided with gas masks but not with protective clothing, HN3 vapor may approach H vapor in potency as a casualty-producing agent, particularly when the genital region is unprotected. HN1 vapor, except possibly on freely sweating men acclimated to hot weather, appears to be definitely inferior to H and HN3. The laboratory findings (see the preceding section) suggest that HN1 vapor would be markedly inferior under cool or temperate conditions.

3. *Evaluation of protective clothing.* The merits and limitations of the CC-2 impregnated and the carbon-containing types of protective clothing are reviewed in Chapters 26 to 30. In brief, the data^{72,73,109,111,113} reveal that CC-2 impregnated clothing offers excellent protection against H, considerable protection against HN3, and relatively little protection against HN1. Thus, in the tropics against troops protected by this clothing, HN1 vapor may be a more potent casualty-producing agent than H. The relative positions of H and HN3 are not known but may not be important because of the high degree of the protection afforded against both agents.

The best experimental types of carbon clothing now available offer protection against such large dosages of H, HN1, and HN3 (presumably also HN2) that differences between the dosages of the agents required to "break" this clothing become of minor consequence.

4. *Protection afforded by ointment.* Prophylactic use of S-330 ointment, and presumably other ointments containing the chloramides available to Allied troops during World War II, offer little protection to skin exposed to nitrogen mustard vapor.¹⁰⁹

6.4.4 Eye-Injuring Action

Numerous observations on the effect of the nitrogen mustards on human and animal eyes demonstrate that HN1, HN2, and HN3 are eye-injurants more insidious than H and more or less comparable with it

TABLE 12. Basic man-chamber tests with H and nitrogen mustard vapors; United States Naval Research Laboratory data.^{110,111}

T = 90 F. Relative humidity = 65 per cent. Exposure time = 60 minutes. All subjects wore gas masks, unimpregnated outer and under clothing, caps, shoes, and socks. The maximum lesions sustained on various parts of the body over a period of approximately a week were graded according to the following numerical scale:

- 0 = No reaction.
- 1 = Mild erythema.
- 2 = Moderate erythema.
- 3 = Intense erythema.
- 4 = a. Erythema with edema.
b. Maceration of axillary skin.
c. Dry scaling of scrotum.
- 5 = a. Vesicle.
b. Numerous pinpoint vesicles.
c. Crusting or ulceration of scrotum or axilla.

Season	Number of men	Vapor dosage (mg min/m ³)	Severity of injury			No. of crusted or ulcerated scrotal lesions
			Neck	Scrotum	Rest of body	
<i>H</i>						
March	6	50	0.3	0.0	0.1	
July	5	50	1.2	0.2	0.5	
March	6	100	1.2	1.2	0.7	
July	5	100	1.6	0.8	0.9	
April	10	150	2.0	0.3	0.7	
July	6	150	3.0	2.2	2.1	
April	10	200	2.2	2.1	1.2	
July	6	200	4.0	3.2	2.4	
April	15	250	2.4	3.2	1.6	
July	6	250	4.2	3.7	2.8	
November	6	300	3.3	0.0*	2.4	
March	5	300	3.4	0.0*	2.9	
<i>HN1</i>						
August	10	100	1.3	1.2	0.3	0/10
August	10	200	3.4	1.4	1.2	0/10
August	10	300	3.3	4.6	1.7	7/10
January	8	300	1.9	0.6	0.3	0/10
January	4	450	1.8	1.5	0.6	0/10
January	6	700	2.2	4.0	0.6	4/6
<i>HN3</i>						
September	8	50	1.8	0.5	0.2	0/8
September	8	100	3.0	1.9	0.8	1/8
September	8	150	4.0	4.0	1.8	6/8
August	8	150	2.0	0.0*	0.2	0/8*
February	6	150	1.5	0.3	0.7	0/6
February	6	250	2.5	1.5	0.8	0/6
February	8	350	4.9	3.6	1.7	6/8

* Subjects wore CC-2 impregnated shorts.

in potency.^{8,44c,d,h,45,56,57,63,64,65,66,69,70,71,81,84,85,88,101,104b,c,106a,112,115,118,123,132,133,134,138,142,150,152,156,174,175,180,184} Propyl-*bis*(β-chloroethyl)amine and isopropyl-*bis*(β-chloroethyl)amine appear to be somewhat less potent.^{44c,81,134}

EFFECTS OF THE VAPORS IN SMALL DOSAGES ON HUMAN EYES

The results of observer tests with H, HN1, HN2, and HN3 as vapors demonstrate that all four agents

are *roughly* comparable on a potency (dosage) basis in eye-injurant action. Provisionally it would appear that HN2 and HN3 may be somewhat more potent than H, and HN1 somewhat less potent, but the differences cannot be considered to have been established with significance (see the following section for animal data). The importance of the human eye data merits their more detailed review as follows.

1. H. Critical summary and review^{112,113} of the four available sets of data^{107a,119,147a,196a} suggest that

50 mg min/m³ ($t < 8$ hours) is the maximum dosage to which unmasked personnel may be exposed without danger of significant eye damage, and that 100 mg min/m³ ($t = 6$ minutes to 7 hours) is the threshold dosage for production of partial disability. Extrapolation from the data leads to the estimate that for offensive purposes 200 mg min/m³ ($t = 6$ minutes to 7 hours) would suffice to produce incapacitating conjunctivitis and blepharospasm, with lacrimation, photophobia, and soreness, and perhaps with some corneal damage, in the majority of men for a period of 2 to 7 days, beginning 3 to 12 hours after exposure. H vapor is somewhat less effective at very short (i.e., 1 to 2 minute) and very long exposure times.

2. HN1.¹⁴² A dosage of 90 mg min/m³ is believed to represent the beginning of the human casualty zone, on the basis of tests in which one eye of each of 21 observers was exposed in a respirator facepiece to 5 l/min of HN1 vapor ($Ct = 37$ to 90, $t = 5$ to 67 minutes). There was no serious change of vision except for three men, exposed to dosages of 41, 56, and 90 respectively, who did not think they could shoot a rifle for 48 hours. Only one of three men exposed to a dosage of 90 was a "casualty" in this sense. There was an average delay of 12 hours in the development of symptoms, which included gritty feeling, lacrimation, photophobia, blepharospasm, headache, blurred vision, conjunctival hyperemia, corneal flecks, epithelial bedewing, and punctate staining with fluorescein. Minor symptoms persisted in one case for as long as 24 days. The conclusions of the report are transcribed verbatim as follows:

- a. A dosage of HN1 of 90 mg min/m³ is probably the beginning of the human casualty zone, but with ocular idiosyncrasy casualties can occur at lesser dosages.
- b. The average interval between exposure and onset of symptoms was 13 hours.
- c. The most common complaint was "gritty" foreign body sensation in the eye.
- d. The most common lesion was flecks of the corneal epithelial surface which disappeared spontaneously in 1 to 15 days. Conjunctival hyperemia occurred almost as frequently.
- e. The most annoying symptom was pain in and behind the eyeball.
- f. Other complaints were lacrimation, photophobia, and blurred vision, although there

was never any reduction in visual acuity or accommodation.

- g. Blepharospasm occurred in only two observers and myosis in only one.
- h. So far as can be judged from the results obtained, the dosage (Ct) of HN1 vapor is a sufficient index to the degree of damage anticipated, even though the exposure time be varied from 5 to 60 minutes (but see below).

3. HN2.¹²³ Dosages of 40 to 55 mg min/m³ ($t = 0.5$ and 10 minutes, respectively) are believed to represent the lowest limits of exposure necessary to produce "disablement" — i.e., certain cases would call for medical aid and, to an extent depending on transport and medical facilities, would be unable to take part in operations for a minimal period of 1 to 2 weeks. This conclusion was based on experiments in which an unstated number of men wearing oronasal masks were exposed in a man-chamber to dosages of 10 to 55 mg min/m³. The performance of additional human experiments at higher dosages was considered to involve an unreasonable risk. There were no subjective symptoms during exposure. From 8 to 15 minutes after exposure lacrimation and a feeling of grittiness under the lids developed. After 6 to 10 hours the following symptoms had set in: lacrimation, photophobia, blepharospasm, and pain in the eyeball severe enough to prevent sleep. At 24 hours the symptoms were similar but the pain had become less severe. There was pupillary constriction, conjunctival congestion, deep ciliary congestion, and threshold edema of the corneal epithelium, but no staining with fluorescein. The condition was resistant to mydriasis with 1 per cent homatropine but pupillary dilatation and relief of blepharospasm was achieved by two applications of 1 per cent atropine. The observers gave their opinion that their efficiency as soldiers would have been seriously impaired from 6 to 10 hours onward. The duration of the symptoms was not stated. The report recommends that a dosage of 70 mg min/m³ be aimed at as a minimum for offensive purposes.

4. HN3.^{104b,c} Of four observers exposed to a dosage of 20 mg min/m³ ($t = ?$), none experienced any subjective symptoms but all showed moderate conjunctival injection. Their corneas were grossly normal but examination with the slit lamp revealed moderate to marked epithelial edema. Of three observers exposed to a dosage of 42 mg min/m³ ($t = 7$ minutes),

TABLE 13. Eye damage produced in rabbits by the vapors of H, HN1, and HN3.^{44h,106a}

Eight animals were exposed to each agent at each dosage. The eye damage was graded according to an arbitrary numerical system^{106a} which took account of changes in the iris, cornea, conjunctivas, and lids. The analytical dosages of the agents were determined by methods adequate to integrate low concentrations over long times.

Exposure time (min)	H		HN3		HN1	
	Dosage (mg min/m ³)	Eye damage	Dosage (mg min/m ³)	Eye damage	Dosage (mg min/m ³)	Eye damage
2	440	20	353	21	485	30
	384	19			439	29
10	330	29	410	23	650	15
	370	24			389	12
60	420	23	418	25	435	12
	434	24			460	10
200	420	17	411	16	530 466	11 10
240	347	21				
360	330	13				

all developed lacrimation, photophobia, and a feeling of grittiness in the eye. They exhibited marked conjunctival injection. Their corneas were grossly normal and did not stain with fluorescein but examination with the slit lamp revealed epithelial edema and slight infiltration of the anterior stroma. One developed moderate edema of the lids. All three were improving both subjectively and objectively on the fourth day after exposure. On the basis of the brief available description it would appear that HN3 produced effects comparable to those found for H in one investigation^{19a,b} and more severe than those found for H in two other investigations.^{119,147a}

The clinical reports of plant accidents indicate that the development of eye symptoms due to the vapors of HN1 and HN3 were delayed for several hours.^{69,174} The same delay was experienced by some workers exposed to HN2 vapor, but others developed eye irritation, lacrimation, and photophobia immediately after exposure.¹⁷⁵

EFFECTS OF VAPORS ON ANIMAL EYES

Although the animal (i.e., rabbit) eye is considerably more resistant to H and nitrogen mustard vapors than is the human eye,^{75,119,142} it may be assumed that the *relative* potencies of the different agents can be determined in animal tests.

The most satisfactory available set of comparative data is summarized in Table 13.^{44h,106a} The results suggest that the rabbit is approximately as susceptible to HN3 as to H. HN1 is probably more potent than H and HN3 at very short exposures (i.e., 2 minutes) but significantly less potent for exposure times of 10 to 240 minutes. The results of less rigorously

controlled earlier work with dogs exposed for 10 minutes suggest that HN1, HN2, and HN3 produce threshold corneal damage at somewhat lower dosages than H; that at low dosages HN3 is the most potent eye-damaging agent, followed by HN1, HN2, and H; and that at higher (but still moderate) dosages the differences among the four compounds are less conspicuous.^{44c}

In tests with relatively large vapor dosages which produced severe ocular injury, it was found that the dosages required to produce equally severe superficial corneal and conjunctival injury were about the same for each of the three nitrogen mustards.^{57b} With equally severe injury to the superficial corneal tissues, however, the damage to the deeper tissues (i.e., iris and ciliary body) was much the greatest with HN2, intermediate with HN3, and least with HN1 and H.^{57b} The severity of the deep ocular effects produced by HN2 make it a particularly dangerous agent from the standpoint of severe and permanent eye injury.

The results of additional studies on the effects of nitrogen mustard vapors on animal eyes are to be found in the following references, some of which contain more or less complete histopathological analyses.^{44d,45,57a,c,65,70,71,84,85,123,138,142}

The clinical and pathological studies with HN2 have been reviewed in detail.⁶³

LIQUID CONTAMINATION OF THE EYE

Tests on animal eyes with small liquid drops (i.e., 0.5 $\pm$ mg) of H, HN1, HN2, and HN3 demonstrate that all the agents produce such severe burns, frequently with permanent loss of sight, that any differ-

ences in potency which may exist are relatively unimportant to an evaluation of their relative merits as offensive agents.^{56b,c,101,123,152,156,180} The observations tend to emphasize the similarity of the lesions produced by H and HN3 and the more severe character of the injury that HN2 produces in the deeper structures of the eye.

The effects of small droplets, and in the wind tunnel of sprays consisting of fine droplets and vapor, have also been studied in animal experiments in order to assess the relative effectiveness of the agents in the initial clouds produced by bursting munitions.^{123,150,152} The results indicate that HN3 may be slightly less damaging, and HN2 slightly more damaging, than H. In any event the differences are not marked.

DECONTAMINATION AND TREATMENT

Decontamination can be effected practically only by prompt lavage of eyes contaminated with the agents in the liquid form. There is some evidence that lavage is of less value with HN3 than with H.¹⁵⁰ Prompt use of dithiocarbamates or of BAL ointment may be of limited value.^{56a,57c,105}

The subject of treatment has been authoritatively reviewed.⁶² The susceptibility to infection of eyes injured by nitrogen mustard and the value of various types of chemotherapy have recently been investigated.^{184c,d}

6.5 RESULTS OF FIELD TRIALS

Field trials with the nitrogen mustards have included tests of the vapor return from contaminated terrain and study of casualties in animals exposed to clouds of liquid drops and vapor produced by bursting munitions. No observer tests have been made to determine the vesicant effects of evolved vapor in the field or the hazard to traversal and occupation which is presented by the liquids on soil and vegetation.

The results of the tests reviewed in Section 6.2.4 attest to the excellent stability of HN3, the probably adequate but marginal stability of HN1, and the questionable stability of HN2.

HN1, HN2, and HN3, as well as H, dispersed from explosive munitions as clouds of liquid drops and vapor can produce profound eye damage and serious, often fatal, respiratory injury in unprotected animals exposed on open terrain (see references cited in Section 6.2.4). However, such trials may have only limited bearing on the general utility of the agents in warfare.

EVOLUTION OF VAPOR FROM CONTAMINATED TERRAIN

Results of field trials (see Table 14) conducted during warm weather at Bushnell, Florida, are available.^{65,66} The tests included both annulus trials and trials with single, statically exploded M47A2 bombs. They lead to the following tentative conclusions.¹¹²

1. When terrain is similarly contaminated with HN3 and Levinstein H, the vapor dosage of H evolved during the first few minutes is five to eight times as great as that of HN3, as would be predicted from the relative volatilities of the agents. With the passage of time the relative dosage of evolved HN3 vapor becomes progressively greater until, after the lapse of sufficient time for the completion of the evaporation process, the total dosages of the two agents become approximately equivalent. The time interval after which the evolved dosage of HN3 attains any specified fraction of the H dosage depends on the meteorological conditions and the size of the liquid drops with which the terrain is contaminated.

2. In trials under semitropical meteorological conditions with single, statically fired M47A2 bombs charged HN3 or Levinstein H, the areas over which toxicologically significant dosages of HN3 vapor were obtained within 4 hours amounted to substantial fractions of the areas over which equivalent dosages of H vapor were obtained (see Table 14).

3. It is estimated⁶⁵ that in large-scale attacks under the semitropical conditions prevailing during the Florida trials the 4-hour vapor dosages obtained from equal expenditures of M47A2 bombs charged HN3 or Levinstein H would be:

Meteorological conditions	4-hour vapor dosages, HN3 as per cent of H
Woods, clear day	45
Woods, clear night	20
Open, clear day	65

4. At the lower surface temperatures characteristic of cool or temperate weather, the times after contamination at which the evolved HN3 vapor dosages would attain the above percentages of the H dosages would be greatly prolonged.

5. Under semitropical meteorological conditions the persistencies of vapor evolution by HN3 and Levinstein H are not *markedly* different. Both are, of course, much less than that of HT (see Chapter 5).

6. HN3 vapor evolved from contaminated terrain in the annulus and bomb trials was proved by bioassay tests to be toxicologically effective. On the basis of the respiratory and ocular lesions produced in rabbits exposed at intervals up to more than 24

TABLE 14. Results of field trials with HN1, HN3, and H; single bomb tests.^{65,66,112}

Agent and test	Bomb	Avg wind speed at 2m (mph)	Average ground temp (C)	Avg temp gradient $T_{2m} - T_{0.2m}$, in the open	Area (artillery squares) within the contours for the stated dosages (Ct 's in mg min/m ³) for 0 to 0 + 4 hour sampling at a height of 12 inches.					
					50	100	250	500	1,000	2,500
<i>Meadow, lapse conditions</i>										
HN1, test 4	M47A2	4.43	23.81	-1.11	0.98	0.61	0.32	0.17	0.09	...
H, predicted	M47A2	4.43	23.81	-1.11	1.01	0.59	0.29	0.17	0.10	...
H, observed	M47A2	4.3	17.0	-1.2	0.81	0.51	0.22	0.13	0.07	...
HN1, test 6	M47A2	4.0	23.0	+0.7	1.72	1.03	0.48	0.27	0.15	0.06
H, predicted	M47A2	4.0	23.0	+0.7	1.77	1.03	0.51	0.29	0.17	0.08
H, observed	M47A2	4.41	21.09	+0.52	2.18	1.22	0.55	0.31	0.17	...
HN3, test 6	M47A2	3.3	35.2	-1.1	0.76	0.50	0.30	0.18	0.11	0.06
H, predicted	M47A2	3.3	35.2	-1.1	1.34	0.79	0.40	0.23	0.14	0.07
H, observed	M47A2	4.8	32.8	-1.32	0.77	0.48	0.25	0.16	0.10	0.06
H, observed	M70	4.2	36.2	-2.24	0.71	0.45	0.23	0.14	0.09	0.05
<i>Forest, lapse conditions</i>										
HN3, test 4	M47A2	1.06	29.3	-1.05	0.57	0.35	0.20	0.13	0.09	0.06
H, predicted	M47A2	1.06	29.3	-1.05	0.92	0.59	0.35	0.24	0.16	0.08
H, observed	M47A2	0.9	27.5	-1.10	1.10	0.72	0.41	0.25	0.16	0.09
H, observed	M70	0.5	26.5	-1.03	0.90	0.67	0.45	0.32	0.22	0.12
H, observed	M70	0.9	28.8	-1.08	0.45	0.30	0.20	0.14	0.10	0.07
<i>Forest, inversion conditions</i>										
HN3, test 5	M47A2	0.5	24.5	+0.3	1.18	0.64	0.24	0.12	0.08	0.05
H, predicted	M47A2	0.5	24.5	+0.3	3.83	2.38	1.21	0.71	0.38	0.19
H, observed	M47A2	0.6	21.5	-1.45	1.16	0.66	0.36	0.23	0.13	0.06
H, observed	M47A2	0.5	19.5	+1.76	4.18	2.78	1.27	0.76	0.20	0.08
H, observed	M70	0.6	25.5	+0.80	2.75	2.02	1.38	0.84	0.47	0.17

hours after exposure, HN3 vapor was significantly more potent than H vapor.

7. When terrain is similarly contaminated with HN1 and Levinstein H in the form of large drops in annulus trials conducted in the open in warm weather, the initial rate of vapor evolution was greater for HN1 than for H, as would be predicted from the relative volatilities, and the 4-hour dosages of HN1 were nearly twice those of H.

8. In the available single-bomb trials in the open under semitropical meteorological conditions the 4-hour dosages of HN1 vapor were approximately equal to those obtained with H in similar tests (see Table 14). Approximately 90 per cent of the total evolved dosage of HN1 vapor had been attained within this time.

9. Analysis of the data indicates that the destruction of HN1 during the explosion or, subsequently, by inactivation on soil and foliage may have been as much as 30 per cent greater than the loss of H. Taking these results in connection with those of British annulus trials¹⁵⁵ which indicated 50 per cent destruction of HN1 on soil, it seems probable that large variations in per cent destruction may be expected, depending on the munition utilized and the character of the terrain upon which the agent is deposited.

Even greater variations might be expected in the case of HN2.

10. HN1 vapor evolved from contaminated terrain in the annulus and bomb trials was proved by bioassay tests to be toxicologically effective. In terms of the respiratory and ocular injuries produced in exposed rabbits, it was somewhat less effective on a dosage basis than HN3 vapor under similar conditions.

6.6 EVALUATION AS WAR GASES

The instability of HN2 disqualifies it from serious consideration for use as a war gas. Isopropyl-bis(β -chloroethyl)amine is also disqualified because its somewhat inferior toxicological potencies are not counterbalanced by other advantageous properties. Thus only HN1 and HN3 remain as potential substitute persistent agents for H. In Table 15 are summarized the properties of H, HN1, and HN3 which bear most directly on an evaluation of their relative merits and limitations.

The judgment of the present reviewers is in accord with the principal conclusions of previous assessments:^{112,118} (1) that HN1 and HN3 do not possess the general utility of H as an offensive agent; and (2) that in so far as incapacitation of masked enemy

TABLE 15. Properties of H, HN1, and HN3 bearing on their potential effectiveness as war gases.

Property	H	HN1	HN3
Storage stability	Good	Satisfactory	Excellent
Stability on explosion of munitions	Good	Probably sufficient	Good
Stability on terrain	Good	Good to poor, depending on the nature and moistness of the terrain	Good
Density (g/ml, 25 C)	1.27	1.09	1.23
Load carried by M47A2 bomb (lb)	69 (pure H) 71 (Levinstein H = 53 lb of active agent)	61	67
Freezing point (C)	14.2 (pure) ca. 8 (Levinstein)	-33 ±	-3 ±
Volatility			
mg/l at 25 C	0.96	2.3	0.12
relative to H, 10-40 C	1	2.2-2.6	0.11-0.14
Median detectable conc. (μg/l)	0.6-1.8	13-17	15 or more
Minimal vapor dosage producing significant eye damage in man (mg min/m³)	100	≥100	≤100
Relative injury-producing effectiveness against masked troops without protective clothing	1	<1, much less except possibly under hot tropical conditions	1 ±
Injury-producing effectiveness of vapor against masked troops with 2 layers of CC-2 impregnated clothing	Ineffective in reasonably attainable dosages	Not known whether casualty production would be feasible	Ineffective in reasonably attainable dosages
Injury-producing effectiveness of vapor against masked troops equipped with clothing containing activated carbon	Ineffective in reasonably attainable dosages	Ineffective in reasonably attainable dosages	Ineffective in reasonably attainable dosages
Relative injury-producing effectiveness of liquid on bare skin	1	$\frac{1}{2}$ - $\frac{1}{4}$	<8
Relative injury-producing effectiveness of liquid through CC-2 impregnated clothing	?	?	?

troops not equipped with chloramide-impregnated clothing is the primary objective in the use of a persistent agent, HN1 and HN3 do not possess the offensive potential of H. At the present time, however, it is pertinent to add a discussion of two additional points.

1. The lack of reactivity of HN1 and HN3 with the chloramides used in the United States and British impregnated clothing of World War II led to the inference that this type of clothing would afford little protection against the vapors of these agents, and that they would therefore be more effective casualty-producing agents than H against troops so equipped.^{112,118} Recent man-chamber tests at 90 F reveal, however, that subjects exposed in 2 layers of

CC-2 impregnated clothing to 5,000 mg min/m³, and in 1½ layers to 1,600 mg min/m³, of HN3 vapor failed to sustain injuries of incapacitating severity.⁷³ Thus CC-2 impregnated clothing affords marked protection against HN3 vapor, although not necessarily so much as against H vapor. The explanation of this unexpected finding is not at hand. On the other hand it has been confirmed that CC-2 impregnated clothing affords little protection against HN1.⁷² However, this lack of protection is at least partially offset by the additional evidence that HN1 vapor is relatively ineffective as a vesicant, except possibly in very hot weather.⁷²

2. It was the intention of the German Army to use HN3 in high explosive-chemical shells. In the

opinion of the reviewers, this means of exploiting HN3 merits careful evaluation. When HN3 is used in this way as a harassing and casualty-producing agent, no other known gases except the Trilons (see Chapter 9) would be expected to approach it in effectiveness. It is believed that in high-explosive bombardments an occasional high explosive-chemical shell charged HN3 and indistinguishable upon detonation from ordinary high-explosive shell would have been used. HN3 possesses the stability to withstand destruction during the explosion of the shell and the lack of odor to escape ready detection except by chemical methods. It is believed that the potential harassing and casualty-producing effects of the vapor slowly evolved from the contaminated terrain might exceed those of the initial cloud. The duration

of danger from the vapor, the time intervals required for the evolution of casualty-producing dosages, and the areas over which effects would be produced would depend on meteorological conditions. As an example of the order of magnitude of the hazard, however, reference may be made to the field trial data reviewed in Section 6.5 and Table 14. It will be noted that in warm weather explosion of a single M47A2 bomb (containing 67 pounds of HN3) resulted within 4 hours in the attaining of a dosage of 100 mg min/m³ of vapor over approximately one-half of an artillery square, and of 250 mg min/m³ over about one-fourth of an artillery square. A dosage of 250 mg min/m³ should more than suffice to produce total disability of several days' duration due to eye injuries, and possibly severe respiratory injury as well.

Chapter 7

ARSENICALS

By Marshall Gates, Jonathan W. Williams, and John A. Zapp

7.1 INTRODUCTION

IN JULY 1917, the Germans not only introduced mustard gas into World War I, but also employed for the first time an arsenical chemical warfare agent, diphenylchlorarsine (DA). Other arsenical agents were employed by the Germans in rapid succession, phenyldichlorarsine (PD) in September 1917, ethyldichlorarsine in March 1918, diphenylcyanoarsine in May 1918, and ethyldibromoarsine in September 1918. Although lewisite and adamsite were not actually used in battle, the Allies were preparing at the end of World War I to use β -chlorovinylchlorarsine (lewisite) and diphenylaminechlorarsine (adamsite), and were seriously considering the use of methylchlorarsine and arsine itself.

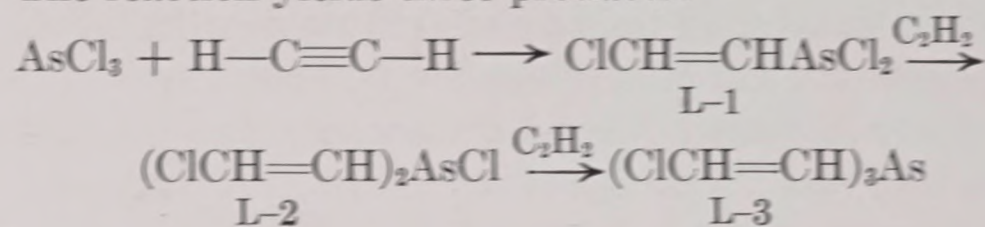
There was a distinct feeling on the part of the Allies that the Germans did not obtain the maximum effectiveness from the arsenicals which they used because of technical difficulties in methods of dispersion, and further that some of the agents which did not receive battle trial (e.g., lewisite and adamsite) might become the most effective agents of their class. In view of this, it was natural that attention again be turned to the arsenical agents at the beginning of World War II. Accordingly, both the British and the Americans carried out extensive investigations on (1) improved methods of preparation of the known arsenicals, (2) the preparation of small quantities of new arsenicals, and (3) the physiological action, toxicology, and assessment of military value of these agents. Although considerable progress was made in the first two categories, none of the arsenical agents proved to offer much promise of success in battle for reasons which are detailed below.

7.2 CHEMICAL SECTION

7.2.1 Lewisite

Lewisite, developed during World War I, is undoubtedly still the best arsenical for gas warfare. (For a summary of work to 1940, see the bibliography.)¹²² The preparation of the agent by the original procedure¹⁰⁶ was complicated and dangerous; it involves the reaction of acetylene with arsenic

trichloride, using aluminum chloride as a catalyst. The reaction yields three products:



When aluminum chloride is used as the catalyst, the very vigorous reaction leads to a mixture in which L-2, L-3, tar, and an explosive material are present with the desired lewisite. The optimum yield of L-1 in this scheme is about 20 per cent.³³⁵⁻³³⁸ It was highly desirable, therefore, to search for other catalysts.

The first work with a catalyst other than AlCl_3 was carried out in Great Britain in 1938,^{286a} where it was shown that acetylene can be made to react directly with arsenic trichloride in hydrochloric acid solution using mercuric chloride as a catalyst. The yield of L-1 was 80-85 per cent based on the arsenic trichloride and 75 per cent on the acetylene. The main drawback to this process was the very corrosive nature of the catalytic solution. A pilot plant operated by the British at Sutton Oak was found capable of producing 10 tons per week of "stripped lewisite," which analyzed: L-1, 83.7 per cent; L-2, 11.5 per cent; arsenic trichloride, 2.8 per cent; solvent (chlorinated hydrocarbon) 2.0 per cent.²⁸⁶ⁱ Work in this country^{139,141,144} showed that a batch process for L using a mercuric chloride catalyst is economically advantageous.

Work on other catalyst systems proved cuprous chloride used in conjunction with ethanolamine hydrochloride to be one of the best, both for batch and continuous operations.^{6,165,185,188,193,200,236f,g,290s}

Although the reaction rate is somewhat slower than with HgCl_2 , the product is cleaner and there is less of a corrosion problem. It was also shown²⁰⁰ that the cuprous chloride process gives 50 per cent more production and 5 per cent greater acetylenation efficiency, and that only one-half the amount of thionyl chloride or phosgene-hydrochloric acid is needed in treatment for sludge removal. A plant operated by this process at Sutton Oak produced 10 tons per week of "stripped lewisite."^{286j}

Many workers recognized the desirability of a con-

tinuous vapor phase process for the preparation of L whereby a mixture of arsenic trichloride vapor and acetylene could be passed continuously over a catalyst. Some degree of success was attained by the use of mercuric oxide suspended on alumina in an all-glass reactor.⁶⁴ With antimony trichloride as an activator for the mercuric oxide catalyst, the conversion was from 30–40 per cent, with yields of 40–60 per cent during the first hour; however, the life of the catalyst was quite short.⁵⁵

Early in World War II, it became apparent that there existed a shortage of pure arsenic trioxide used in the preparation of arsenic trichloride for lewisite production. Consequently two programs were inaugurated: (1) the conversion of crude arsenic trioxide to arsenic trichloride; and (2) the use of arsenic trichloride containing impurities in lewisite production by the mercuric chloride process. In a study of the latter problem it was shown that arsenic trichloride from crude arsenic trioxide can be used directly in a lewisite plant. Incidentally it was indicated that slightly higher absorption rates were obtained when either 2 per cent antimony trichloride or 1 per cent ferric chloride had been added to the arsenic trichloride.⁴² This demonstration led to the observation that, when antimony trichloride is included in the catalyst layer in the mercuric chloride process, the output of lewisite is materially increased.⁵⁵ In pilot plant operations, it was found that, when the same volume of SbCl_3 -containing catalyst (26 per cent SbCl_3 added to the standard HgCl_2 catalyst) is used in the standard HgCl_2 batch process, the time required for acetylenation is reduced by about 40 per cent, whereas the amount of Hg present is 72 per cent of normal.²⁰⁵

The problem of using crude white arsenic in the production of arsenic trichloride was investigated first on a laboratory scale and then in a pilot plant.^{50,57,196,197} With the use of three different raw materials, one of them containing only 51 per cent arsenic trioxide, for reaction with sulfur monochloride, yields of 95 per cent based on both arsenic and chlorine were obtained in pilot plant runs. With this experience as a background the process was transferred to the Pine Bluff Arsenal,⁶² where about 80 tons of specification-grade arsenic trichloride was produced from two lots of crude arsenic trioxide recovered from ore of the Gold Hill, Utah, deposit. A yield of 95 per cent was obtained based on the arsenic content of the crude arsenic trioxide. Practically all of the arsenic trichloride produced in the

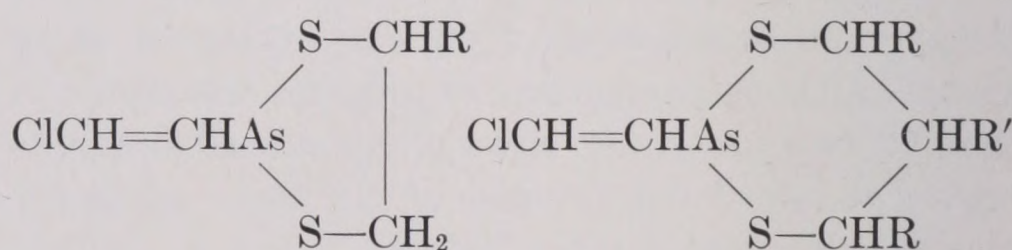
experimental runs was consumed in the lewisite plant with satisfactory results.

It was also demonstrated that arsenic trichloride of high purity can be prepared from either refined white arsenic or from low-grade arsenic crudes and hydrogen chloride in yields of 97 to 99 per cent based on the arsenic content of the raw materials.⁵¹

In connection with the use of lewisite as a chemical warfare agent it was necessary to study its corrosive effect on shell steel. It was shown that plant-grade lewisite produced by the mercuric chloride process is practically without action on shell steel (No. 1045) and may be stored in such steel for long periods of time at tropical temperatures with insignificant corrosion.²⁶ Under these conditions no pressure is developed and no deterioration of the lewisite results. Phosphorus pentoxide may be used to decrease corrosion slightly, to eliminate the slight rust formation, and to prevent the increase in moisture content under damp storage conditions.

Through other studies it was found that a 1/1 mixture of lewisite and Levinstein mustard is far more corrosive than either constituent alone, and that a 1/1 mixture of lewisite and thiodiglycol mustard is only one-tenth as corrosive as the other mixture.^{29,31} The conclusion reached, therefore, is that pure mustard must be employed if mixtures of it with lewisite are to be used in chemical warfare.

Several investigations were made in order to discover agents other than BAL (2,3-dimercaptopropanol-1) which might serve to detoxify lewisite or act as antivesicants for it. In a study of the reaction products of lewisite and six different dithiols¹⁰ it was found that the properties of the compounds are best explained by cyclic formulas of the types:



In a study of the reaction of lewisite with thiols, alcohols, and amines it was shown that the competitive rates of formation, or the stability at equilibrium, or both, of bonds involving arsenic are in the order $\text{As}-\text{S} > \text{As}-\text{O} > \text{As}-\text{N}$; hence α -dithiols appear to be the most satisfactory reagents for detoxification of lewisite.^{30,33}

It has been shown that urea peroxide reacts readily with lewisite to give a nonvesicant product.¹⁴ However, a careful investigation failed to reveal a suitable

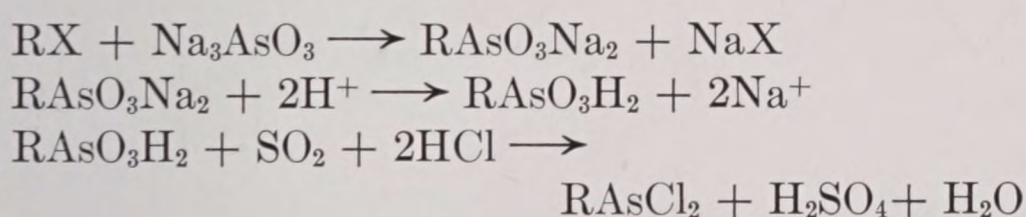
method of stabilizing urea peroxide at 60 C for field use.²⁴ Other peroxides were studied and it was found that 10 g of a 1/1 mixture of sodium perborate monohydrate and sodium dihydrogen phosphate monohydrate, either in the form of a tablet or as a powder dissolved in 50 ml of water, gives a solution equivalent in active oxygen content to a 3 per cent hydrogen peroxide solution. The conclusion was reached that BAL if quickly applied is somewhat more effective as a preventive for lewisite burns than the perborate-phosphate mixture; however, the latter is nontoxic.⁵²

7.2.2 Aliphatic Arsenicals

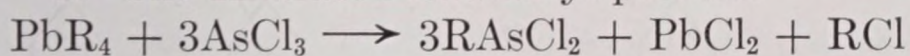
Aliphatic arsenicals in wide variety have been prepared for testing as candidate chemical warfare agents. Major emphasis was placed on alkyldichlorarsines, as it was thought for a while that members of this series might show toxicity equal to that of lewisite and at the same time exhibit greater chemical inertness, particularly in reactions with water. However, it was finally established that *n*-amyl-, isoamyl-, and *n*-hexyldichlorarsine, for example, undergo the same general reactions as lewisite and react at approximately the same rate.³⁵ There is an *apparent* difference in the behavior of the alkyldichlorarsines as compared with lewisite in that the former do not liberate a gas when treated with sodium hydroxide and the alkylarsine oxides remain in solution longer than does lewisite oxide.

The *alkyldichlorarsines* have usually been prepared by the use of one of the following three schemes:

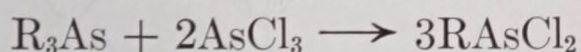
1. The Meyer reaction.



2. The Kharasch lead alkyl process.



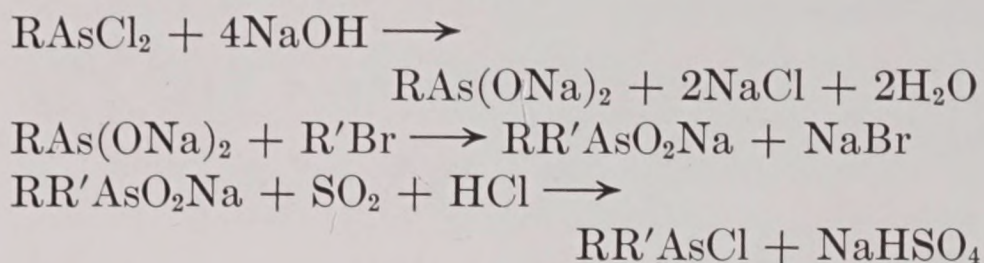
3. From arsenic trichloride and tertiary arsines.



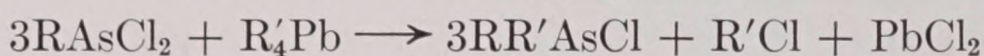
The Meyer scheme is the one most frequently used.^{1,5,25,28,32,39,58,129,290j} It becomes less efficient with the higher alkyl halides, such as heptyl bromide. The Kharasch process is particularly good for the preparation of ethyldichlorarsine in view of the availability of tetraethyllead.⁴⁸ The suitability of the process for large-scale production has been demonstrated by pilot plant operations in which the reaction went readily and smoothly giving a 90 per cent yield.⁵⁶

For the preparation of *dialkylchlorarsines*, four principal routes have been followed.

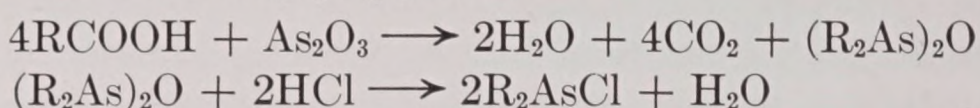
1. The Meyer reaction.



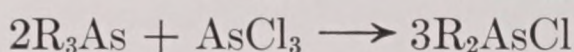
2. The Kharasch lead alkyl process.



3. The cacodyl process.



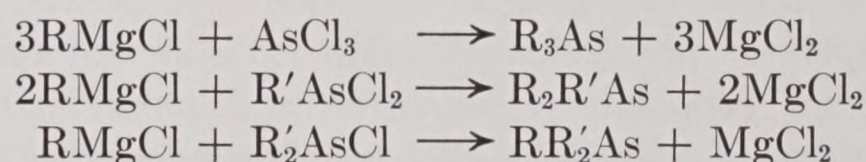
4. From arsenic trichloride and tertiary arsines.



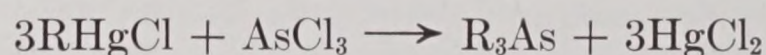
Here, as in the case of the alkyldichlorarsines, the Meyer reaction scheme is the one most commonly followed.^{1,5,28,32,39,58} However, work on the cacodyl process^{8,17,131} has resulted in a marked improvement in this classical reaction. The improvement is in the form of a continuous catalytic process wherein vapors of the acid and arsenic trioxide are passed over an alkali salt on a pumice support. Although this process was previously identified only with the production of dimethylarsine derivatives, it has been demonstrated that higher homologs may be prepared in fair yield.¹⁷

In the preparation of *tertiary arsines*, three general reaction schemes have been used:

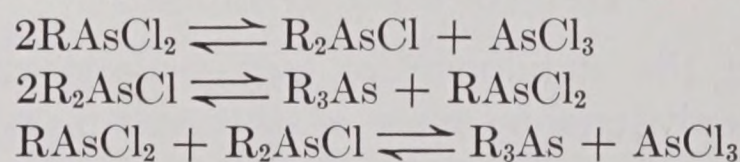
1. Reaction of Grignard reagents with AsCl_3 , RAsCl_2 , or R_2AsCl .



2. Reaction of alkylmercuric chloride with arsenic trichloride.



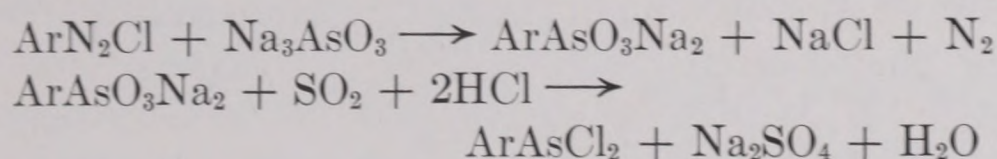
3. Disproportionation of RAsCl_2 or R_2AsCl .



It should be noted that all these methods are laboratory procedures and that no large-scale preparation of an aliphatic tertiary arsine has been attempted.^{1,5,39,58}

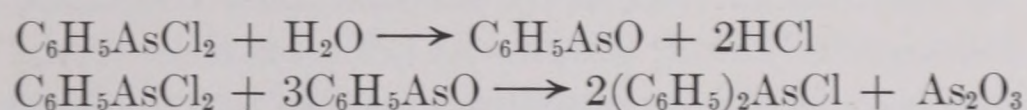
7.2.3 Aromatic Arsenicals

The standard approach to an aromatic arsenical is the Bart reaction between an aryldiazonium halide and sodium arsenite:

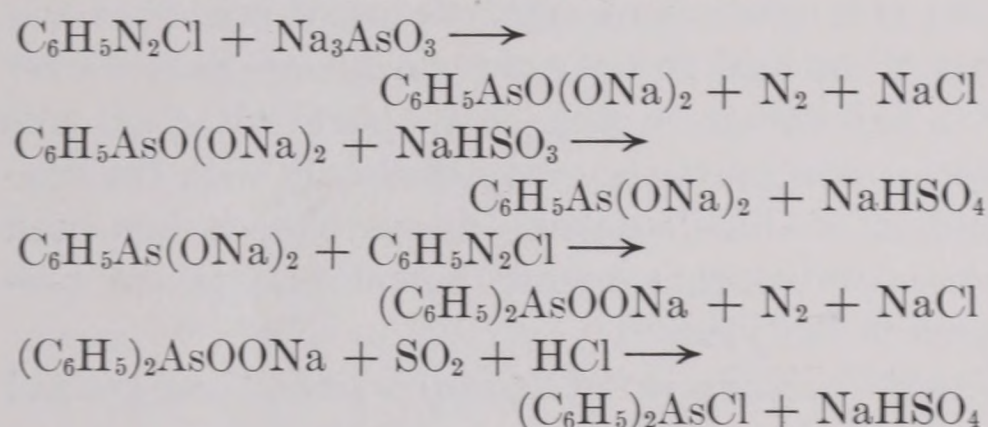


Many aromatic arsenicals desired in the toxicological testing program have been prepared in this manner.^{5,58,301} However, the only aromatic arsenicals produced on any sizable scale during World War II are diphenylchlorarsine (DA) and diphenylcyanoarsine (DC). Considerable attention was devoted by the British to process development studies of those compounds.^{289,290,307} They carried out laboratory and large-scale tests on two processes for DA preparation:

1. The Pope-Turner process.



2. The double diazotization process.



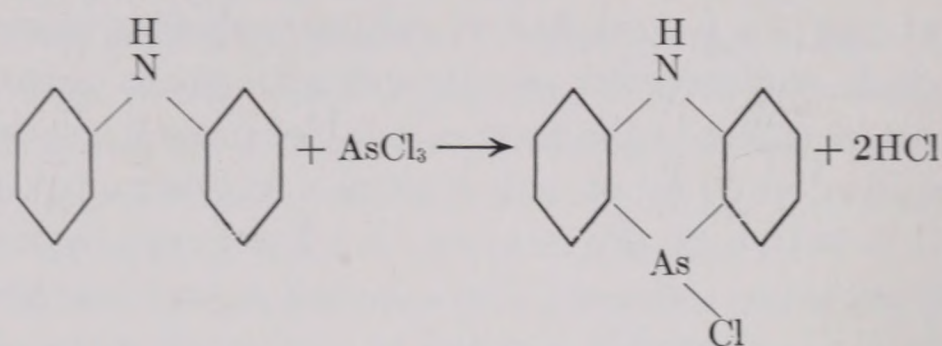
A considerable improvement in the Pope-Turner process was effected by the British workers,²⁹⁰ who worked out the proper conditions for partial hydrolysis of phenyldichlorarsine to a stoichiometric mixture of phenylarsine oxide and phenyldichlorarsine (3/1 mixed oil), which, when heated to 240–250 C, was converted to DA in good yield. DA is readily transformed to DC by reaction with 30 per cent aqueous sodium cyanide at 35–40 C.²⁹⁰

7.2.4 Heterocyclic Arsenicals

From the standpoint of large-scale preparation work, only one member of this group, adamsite (DM), was considered important during World War II. However, representatives of several other heterocyclic types were prepared for toxicity testing.

Adamsite is still prepared by the standard procedure worked out during World War I and involv-

ing the reaction of diphenylamine with arsenic trichloride:

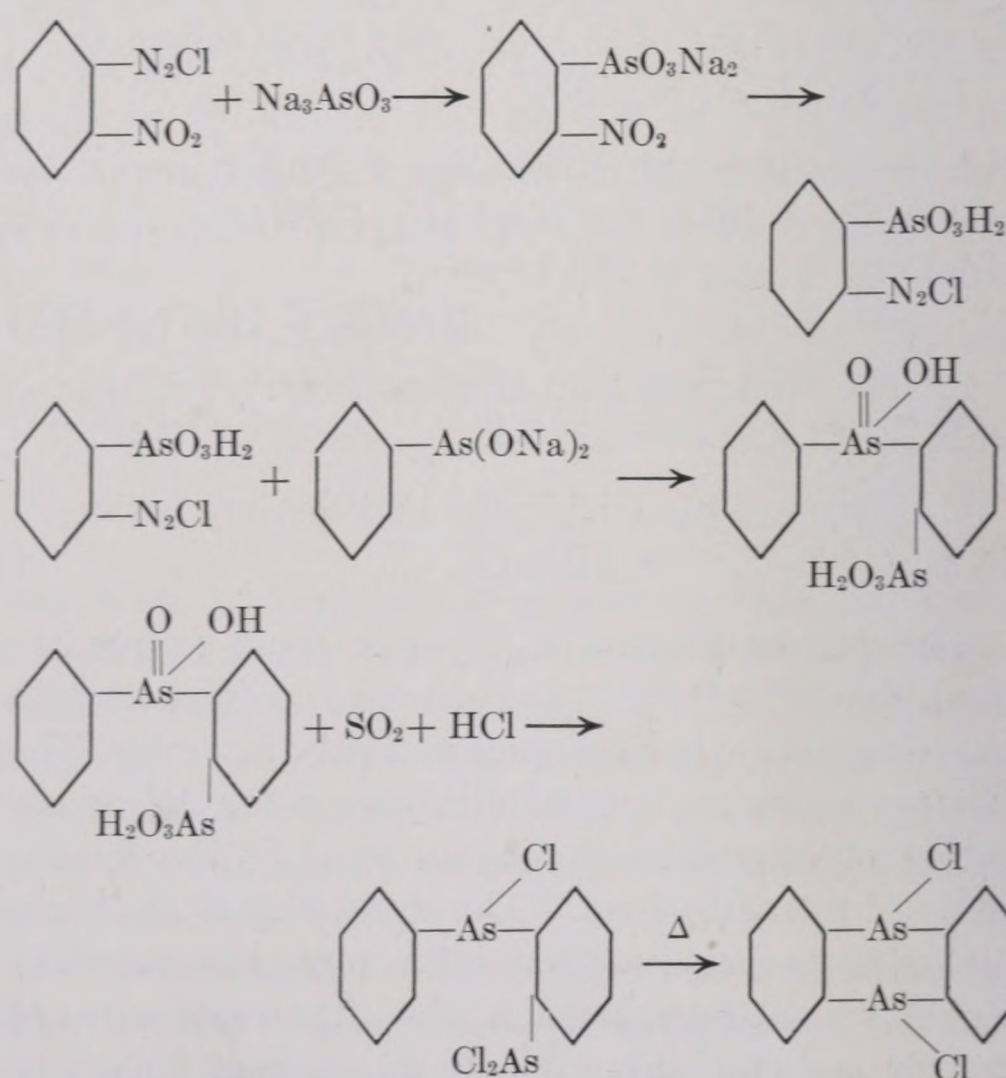


It has been shown³ that a considerable part of the arsenic trichloride called for in this equation may be replaced by the less expensive arsenic trioxide without a sacrifice in yield.

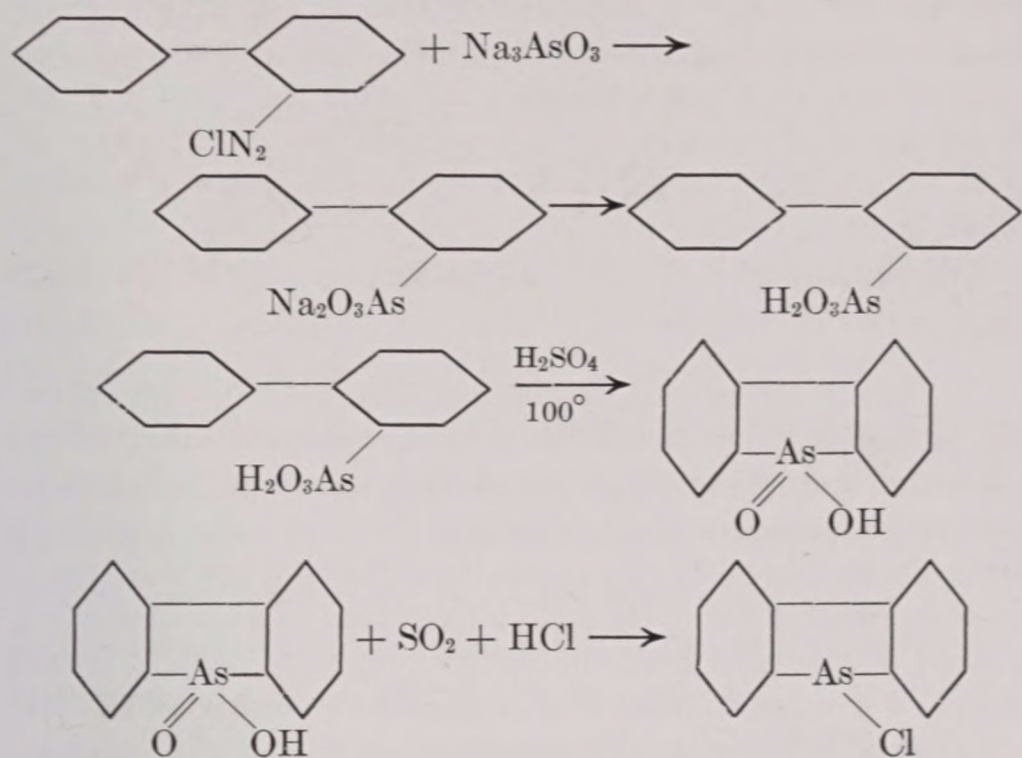
Furan arsenicals were studied both in this country and in Great Britain.^{25,301a} They were prepared by reacting α -chloromercurifuran with arsenic trichloride to give trifurylarsine. From this tertiary arsine the mono- and di-furyl-chlorarsines were made by reaction with arsenic trichloride. Similarly thiophene arsenicals were made from α -thienylmagnesium bromide and arsenic trichloride¹ and pyridine arsenicals were obtained from 3-aminopyridine by the Bart reaction.⁵⁸

Other miscellaneous heterocyclic arsenicals prepared for toxicity testing include 5,10-dichloro-5,10-dihydroarsanthrene,^{4,307b,i,j,k,m} dibenzarsinole chloride,^{5,307b} and 10-chloro-9,10-dihydroarsacridine.^{58,307i,j,k,m,n} The preferred methods of preparation are illustrated by the following equations.

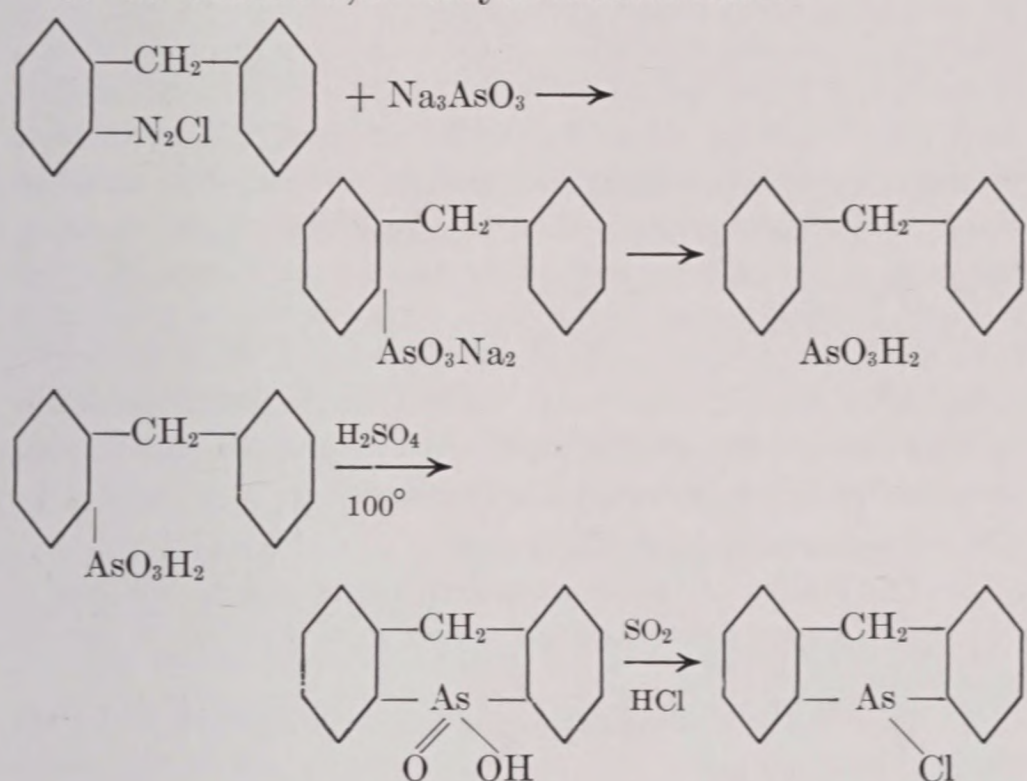
1. 5,10-Dichloro-5,10-dihydroarsanthrene.



2. Dibenzarsinole chloride.



3. 10-Chloro-9,10-dihydroarsacridine.



7.3 PHYSIOLOGICAL SECTION

7.3.1 Lewisite

When the United States became actively involved in chemical warfare during World War I, high hopes were held for a new agent, β -chlorovinylchlorarsine, which was prepared and suggested as a candidate agent by Capt. W. Lee Lewis in 1917. On the basis of relatively meager laboratory data it was decided to produce this agent, Lewisite (L), in quantity and to use it in battle. A shipment was on its way to Europe when the war ended in November 1918.

During 1918, and particularly during the latter half of the year, the toxicological properties of L were studied intensively in various laboratories of the Chemical Warfare Service. The data obtained during this period are well summarized ¹²⁴ and will not be discussed in detail in this report. However, two

reports ^{224,225} issued in 1919 are particularly interesting in that they not only summarize the toxicological data acquired during the war period but also attempt to assess the military value of L as a chemical agent. Since the conclusions of 1919 offer a convenient starting point from which to consider the later developments which took place in the interval between wars and during World War II, these conclusions will be briefly stated.

The effects of liquid L on the skin were studied in detail on dogs and rabbits. ²²⁴ It was felt that L was definitely more damaging to the skin than H and that the danger of systemic poisoning from L was considerably greater than with H. It was concluded that, if man were as susceptible as dogs to systemic poisoning from L, the minimum lethal dose for man would be 1.4 ml distributed over an area of 5 square inches for an individual of average size.

No systematic study of the effect of liquid L on human skin was carried out. However, it was stated that: ²²⁵

Laboratory workers who have been accidentally burned with liquid L have given strong evidence for the greater effectiveness of this substance in man than liquid H. The L lesions develop with extreme rapidity, are painful and associated with definite constitutional symptoms. The lesion is not confined to the skin, but extends to the deeper tissues. In healing, dense scar tissue forms, the skin loses its flexibility and contractures may develop. With liquid H skin burns in man, pain is less or absent, there are no constitutional symptoms, the amount of skin destruction is less, and healing occurs without extensive scar formation, formation of contractures, or permanent disability.

In view of the divergence of these views from those currently accepted, it is well to bear in mind that these were accidental burns and hence were probably treated, that the accepted treatment at the time was application of 5 per cent sodium hydroxide to the lesion for a period of 30 minutes, and that sodium hydroxide itself in that strength produces a very destructive skin effect.

The effects of L vapor on the skin were studied with dogs, rabbits, and man, and are summarized in Table 1. ²²⁵

TABLE 1. Approximate concentration to produce skin lesions in 30-minute exposure.

	Rabbit	Dog	Man
Lewisite (L)	0.025 mg/l	0.050 mg/l	0.200 mg/l
Mustard (H)	0.200 mg/l	0.050 mg/l	0.025 mg/l

The comparison indicated a lower sensitivity of man toward L vapor than toward H. The degree of

protection afforded by ordinary wet and dry clothing against L and H vapor was also studied.

It was concluded:²²⁴

An approximate concentration of .200 mg/l (of L) is necessary to produce skin lesions in man on exposure of one-half hour. To be effective on parts of the body covered with clothing, it would be necessary to raise this concentration from three (3) to one hundred (100) times, or approximately to a concentration of .600 to 20.0 mg/l . . . So far as the concentration required under field conditions to produce cutaneous lesions in man, H should be regarded as from eight (8) (on unprotected skin) to a thousand (1000) times (a single layer of wet wool) more effective than L.

The eye effects of L vapor were studied on rabbits and dogs. As with skin effects, it was found that rabbits were more susceptible to L vapor than were dogs, and a comparison with H revealed that the relative susceptibility of the species toward L and H vapor paralleled that of the skin effects. The data are summarized in Table 2.²²⁵

TABLE 2. Approximate concentration necessary to produce eye lesions in 30-minute exposure.

	Rabbit	Dog	Man
Lewisite (L)	0.001 mg/l	0.020 mg/l	
Mustard (H)	0.050 mg/l	0.020 mg/l	0.001 mg/l

The statement was made:²²⁵ "If we may be allowed to infer or judge of the susceptibility in man without having an actual determination, the conclusion would be that the eye of man is less susceptible to L than to H, but such a conclusion can never convey the conviction as one based on actual determination." No experiments involving the effects of liquid L on the eye were reported.

The respiratory effects of L vapor were studied on dogs and compared with the effects of H vapor, it being found that the dog was approximately twice as susceptible to L as to H. It was pointed out²²⁵ that the concentration necessary to produce death in man on respiratory exposure is not known in the case of either H or L, but that "in the light of our present knowledge we can only conclude that on respiratory exposure, L is to be regarded as approximately twice as effective as H as determined by the concentration necessary to kill. This conclusion, as applied to man, must be made with reservation due to deficiency of data."

With respect to the relative military value of L and H, it was stated:²²⁵

In attempting a comparison of the relative military value of the substances H and L, we meet with the fact that while with the former substance we have a very large experience

from the laboratory, the experimental field and the field of war, our knowledge of the latter is confined entirely to data from the laboratory. The abrupt cessation of experimental work at the American University following the signing of the Armistice in November 1918, prevented the carrying out of field tests with L, preparations for which were already under way.

In summary of the situation in 1918 it was stated:²²⁵

We regard the laboratory data as offering strong support for the probability that L will prove to have great military value. Its actual value can only be definitely determined, however, by further experimental data, especially those obtainable by field tests. It would furthermore seem clear that the usefulness of L in war would differ quite widely from that of H. Little effect should be expected from the vapor when used against troops supplied with an efficient mask equipment, because of the low skin vapor toxicity and the resistance of clothing to penetration of the vapor. This is the condition, on the other hand, in which H has been found most effective. The usefulness of L would be confined to the effect of the substance reaching troops in the liquid phase (splash or mist) by their coming in contact with contaminated material, the influence of the hydrolytic products in contaminating the ground and objects, and the respiratory effects and possibly the eye effects of the vapor in the case of troops unprotected by mask equipment. In those respects L offers many advantages, so far as can be concluded from the data at hand, over H. We feel that L offers sufficient promise to warrant the most careful further consideration. Data which are not at present obtainable and which are most desirable in this connection are as follows:

1. The keeping qualities in steel.
2. The ability of the substance to withstand detonation.
3. The vapor concentration which it is possible to secure and maintain in field tests.
4. The vapor concentration necessary to produce eye lesions in tests on man.
5. The relative importance of burns by liquid H and vapor of H in actual warfare.

In conclusion we wish to repeat: We believe that L will not replace H in warfare, and that in any plans for military operations the production and utilization of H should remain one of the most important propositions. While very promising, the military value of L remains to be established.

In the interval between 1919 and 1940 relatively little research on the toxicology of L was carried out by the Chemical Warfare Service, with the exception of a detailed study which was published in 1923.¹¹⁵ This report has been critically reviewed¹²⁴ and will not be discussed in detail, although a few of the results will be mentioned later in the present report. It was concluded¹¹⁵ that L is superior to H in that it gave deeper and more severe burns as well as systemic disturbances leading to death, but the difficulty of setting up effective vapor concentrations was recognized.

Following the publication in the open litera-

ture^{335,337} of information that L had been seriously considered by the Americans as a war gas, the agent was studied in the laboratories of other nations. The published German reaction was unfavorable. The compound was tested in Germany in 1916³³² and the conclusion reached that it was not reliable as a war gas because its toxic effects were less lasting than those of mustard and the irritant effects were so marked that men would be warned in time of its presence. The opinion was offered³⁴⁰ that the Americans were spared a great disappointment by being unable to use L in World War I. A series of experiments was carried out³³⁹ in which the effects of relatively large doses (one to two drops from an ordinary eye dropper) of H and L on human skin were compared. These experiments, published in 1932, led to the conclusion that L was inferior to H in producing skin injury and that its potentialities as a war gas have been greatly overrated. In reference to the calculation^{224,331} that 1.4 ml of L applied to the skin of a man should be the approximate minimum lethal dose, it was asserted that this amount was applied repeatedly to the skin of human beings without giving evidence of systemic intoxication.³³⁹ The Japanese used a 1/1 mixture of H and L against the Chinese at Ichang in 1938, but subsequent information obtained by the interrogation of Japanese officers revealed that the L was added mainly to lower the freezing point of the H.

The value of L as a chemical warfare agent still remained to be established in 1941. The published German opinions were looked upon with distrust, and, as in World War I, the United States undertook the quantity production of L. The discrepancies in the literature as to the toxicological effects of L had to be resolved and intensive research was carried out both in the United States and Great Britain.

PROPERTIES OF LEWISITE

Plant run L is usually dark brown in color and possesses an odor reminiscent of geraniums. Both the color and odor are due to impurities, which can be removed if the extra effort involved is considered worth while. *Cis*- and *trans*- isomers exist which have almost identical toxicities.¹⁷⁶ L freezes at -18.2°C to 0.1°C , depending on the purity and isomers present. The density of liquid L is 1.886 at 20°C , whereas the density of the vapor is 7.1 compared to air. The volatility of L is greater than that of H and increases somewhat less rapidly than that of H with increasing temperature. The following data

for L are calculated from the vapor pressures;²⁰⁷ comparative data for H are also given.⁹⁹

Temperature C	Volatility (mg/l)		Ratio L/H
	L	H	
0	1.06		
10	2.23		
15	3.29	0.41	8.0
20	4.48	0.65	6.9
25	6.14	0.96	6.4
30	8.62	1.39	5.1
35	11.32		
40	15.75	2.82	5.6

L is fairly stable on storage in glass or steel but is degraded to a considerable extent on detonation.¹⁷⁴

The reaction of L with BAL and certain related dithiols^{300f-300r,315} to form nontoxic complexes has assumed great importance in the treatment of L lesions and of arsenical poisoning from L or other sources.

The chemical properties which most sharply limit the usefulness of L as a chemical warfare agent are the ease with which it reacts with (1) water and (2) alkalis. In contact with water or moist surfaces, lewisite is readily hydrolyzed to the oxide which, although mildly vesicant, is nonvolatile and insoluble in water. Since L "precipitates out" in contact with moist surfaces it is impossible to maintain high vapor concentrations in humid atmospheres. Alkalis decompose L rapidly at ordinary temperatures, and even alkaline soil²¹⁵ rapidly destroys the liquid and imposes a further limitation on its use as a ground contaminant. The maximum efficiency of L is only attained, therefore, under conditions of low temperature or low humidity, both of which minimize hydrolysis, and on dry nonalkaline terrain.

PHYSIOLOGICAL ACTION

Lewisite Vapor. The qualitative effects of L on the eyes, skin, and respiratory tract have been described in the open literature^{218,330} and have also been recently summarized.²¹⁶ They may be very briefly restated as follows:

1. Eyes. L vapor is extremely irritating to the eyes, causing pain, lacrimation, and blepharospasm. The lacrimation and blepharospasm protect in a large degree from further exposure to the vapor but if the *Ct* is sufficiently high the irritation and pain persist and after a few hours are followed by edema of the eyelids and conjunctivitis. Permanent damage is, however, apt to result only from very high concentrations difficult to achieve in the field.

Liquid L is capable of causing severe damage to the

eyes. Pain, lacrimation, and blepharospasm appear immediately, and are followed by edema of the lids, iritis, and conjunctivitis. In severe contamination, ulceration, necrosis, and secondary infection may lead to blindness or to permanent impairment of vision.

2. Respiratory tract. L vapor is irritating to the nasal passages and produces a burning sensation followed by profuse nasal secretion and violent sneezing. On prolonged exposure coughing results and large quantities of frothy mucus may be brought up. The effects of L vapor are so prompt and striking that men usually mask before enough of the compound is inhaled to produce serious injury. However, in experimental animals exposed to vapor in a gas chamber, injury to the respiratory tract is essentially similar to that produced by mustard. Edema of the lung is often more marked and is frequently accompanied by pleural fluid.²¹⁸

3. Skin. L vapor usually produces no more than erythema of the skin, although if the skin is hot and dry and the vapor concentration is high, small, shallow, turbid blisters may develop and may coalesce to

form large vesicles. Such conditions would seldom be realized in the field.

Liquid L on the skin produces an immediate stinging sensation which fortunately warns of its presence. If L is allowed to remain on the skin for 5 minutes, the site of application assumes a cooked appearance, somewhat resembling that from an acid burn. Erythema develops in a short time around the site of contamination and is followed by vesication of the entire erythematous area. L can penetrate the skin, subcutaneous tissue, and muscle, causing extreme edema and necrosis.

The fluid contained in vesicles produced by L tends to be more opaque than that found in mustard blisters, although it is frequently impossible to distinguish L vesicles from mustard vesicles by their appearance.

The fluid from an L blister contains 0.8 to 1.3 γ of arsenic per cubic centimeter, equivalent to 2.5 to 4.0 γ of original L.¹³⁷

4. Systemic effects. The absorption of a sufficient amount of L through the skin of dogs may lead to death within 24 hours and usually within 10 hours.

TABLE 3. Toxicity of L vapor. (All figures are $L(Ct)_{50}$ in mg min/l, exposure time = 10 min, observation period 10 days, except as noted.)

Species	Total exposure	Inhalation only exposure	Body only exposure
Mouse	0.9-1.4 (nom.) ⁷⁷	1.4-1.5 (nom.) ⁷⁷	1.2-1.9 (nom.) ⁷⁷
Mouse	2.8 (nom.) ²⁷	1.6 (nom.) ^{82e}	0.3 (nom.) ^{82e}
Mouse	1.5 (anal.) ⁴³	1.5 (anal.) ^{82e}	7.0 (nom.) ⁴⁵
Mouse	2.5-2.8 (nom.) ¹⁷⁶		
Mouse	0.5 (anal.) ^{258,*}		
Rat	1.5 (anal.) ^{258,†}		20.0 (nom.) ⁴⁵
Rat	0.58 (anal.) ^{258,‡}		
Guinea pig	1.0 (anal.) ^{258,*}		20.0 to 25.0 (nom.) ^{45,§}
Guinea pig	0.47 (anal.) ^{258,‡}		
Rabbit	1.2 (anal.) ^{258,}		15.0 (nom.) ⁴⁵
Rabbit	1.5 (anal.) ^{258,¶}		
Goat	1.25 (anal.) ^{258,**}		
Cat			30.0 (nom.) ^{45,††}
Dog	1.4 (nom.) ^{115,§§}		30.0 (nom.) ^{82e,‡‡} 40.0 (nom.) ⁴⁵

* 9- to 14-min exposure. 21-day observation period.

† 9- to 25-min exposure. 21-day observation period.

‡ 60- to 180-min exposure. 21-day observation period.

§ 10- to 40-min exposure.

|| 7.5- to 13-min exposure. 21-day observation period.

¶ 60- to 310-min exposure. 21-day observation period.

** 100- to 255-min exposure. 21-day observation period.

†† 30- to 45-min exposure.

‡‡ 30- to 60-min exposure.

§§ ($Ct = 1.32$ for $7\frac{1}{2}$ -min exposure and 1.44 for 15-min exposure, 93-hour observation period. The report states that concentrations were determined both as nominal and analytical but only one set is given and it is not characterized.)

Note. Nom. = nominal concentration; i.e., concentration calculated from the amount of L volatilized, the flow rate, and the duration of flow.

$$\text{Nominal concentration} = \frac{\text{Amount volatilized (mg)}}{\text{Flow rate (l/min)} \times \text{Time (min)}} = \text{mg/l}$$

Anal. = analytical concentration; i.e., concentration determined by sampling and chemical analysis of the atmosphere.

A few hours after application, the dogs show evidence of severe intoxication and appear almost moribund. Death apparently occurs from an intoxication which interferes with certain vital processes without producing sufficient anatomical lesions for complete characterization of the immediate cause of death. A frequent accompaniment of systemic intoxication is a change in capillary permeability which permits loss of sufficient fluid from the blood to result in hemoconcentration and profound shock. The blood volume of dogs was observed ²²⁴ to fall as low as 3.9 per cent of body weight in burned animals (normal = 9.7 per cent).

Nonfatal cases may develop a hemolytic anemia, focal necrosis of the liver, and some injury to the intestinal mucosa.

Toxicity. There is no disagreement over the fact that L is a highly toxic compound and that it can produce the physiological effects which have been described. In order to evaluate the usefulness of L as a chemical warfare agent, however, several things must be known. These are:

1. What dosages of L are required to kill men or at least to make them casualties?
2. Can these dosages be attained in the field with a reasonable expenditure of munitions?
3. How easily can the soldier protect himself against the effects of L?
4. Are the results obtainable through the use of L in the field likely to be better or worse than those obtainable with the standard vesicant agent, H?

TOXICITY DATA

The answer to (1) can only be approached experimentally through studies on animals. The toxicity of L vapor toward animals of different species is shown in Table 3. The $L(Ct)_{50}$ of L vapor for man is unknown, but may be estimated (from the data of Table 3) to be of the order of 1.2–1.5 mg min/l (analytical). The $L(Ct)_{50}$ for body exposure only has been estimated to be of the order of 100,⁴⁵ on the basis of animal experiments and with the assumption that the absorption of L through the skin is a function of the ratio of surface exposed to body volume.

The toxicity of liquid L applied via the skin for animals of different species is shown in Table 4. On the assumption that man would be as susceptible as the dog, it was calculated in 1919 ²²⁴ that the LD_{50} for a 70-kg man would be of the order of 1.4 ml of L applied over an area of 5 square inches of skin. It is stated, however, that doses of 1.4 ml can be applied

TABLE 4. Toxicity of lewisite by skin application.

Animal	LD_{50} (mg/kg)	Reference
Mouse	15	87 (Cited by Smith)
Rat	24	300f
Rat	15	318
Rat	24	249
Rat	20	318
Rabbit	5	318
Rabbit	6	249
Rabbit	6	133
Guinea pig	12	249
Dog	38	224
Dog	ca. 70	295a
Goat	24	241
Goat	10	217

repeatedly to men without eliciting any clear-cut symptoms of arsenical poisoning.³³⁹ The LD_{50} for man is probably much greater ^{295a} than the 40 mg/kg sometimes assumed. A case is reported ²²³ in which a worker at Pine Bluff Arsenal suffered accidental lewisite burns over 20 per cent of his body surface (mostly on the legs). He showed an anemia 10 to 15 days after the burn, but no clear-cut signs of systemic arsenical poisoning. It appears, therefore, that man is not nearly so susceptible to systemic arsenical poisoning from skin contamination with L as was originally believed.

The toxic dose of L when administered parenterally is much lower than that required by skin absorption. For example, the LD_{50} for rabbits is stated in one British report ²²⁷ to be 2 mg/kg by either intravenous or subcutaneous injection, and in another ²⁴⁹ to be 0.5 mg/kg by intravenous injection. The intravenous LD_{50} for dogs was found to be 2 mg/kg as compared to 38 mg/kg by skin absorption.²²⁴ Two mg/kg, injected intraperitoneally, has been given as the minimum fatal dose for guinea pigs.^{295a}

It is difficult to see, however, how the enhanced toxicity by parenteral administration can be utilized in warfare.

Casualty production by L may result from the action of the vapor on the respiratory tract, or of the vapor or liquid on the eyes and skin. Assuming that men will be masked, the probabilities of casualty production from the inhalation of vapor are small. Relative to the eyes, it has been shown that for L to produce moderate corneal damage in dogs a vapor Ct of 2.8 mg min/l (nominal) is required; whereas a destructive lesion is produced by a Ct of 5.5 (nominal).^{82f} Analytical concentrations in the above experiments were approximately 50 per cent of the nominal so that an analytical Ct of the same order as

the $L(Ct)_{50}$ by inhalation is required to produce moderate eye damage. Since the immediate response of the eye to L vapor is lacrimation and blepharospasm, both of which protect against further exposure, serious eye casualties from L vapor are not to be expected in conscious men.

Liquid L in the eyes is capable of producing destructive lesions. It has been estimated²⁷⁶ that a drop 170 μ in diameter in the eye of a man would make him a casualty for over a week unless immediately treated. A 0.1-mg drop in the rabbit eye caused perforation of the cornea in approximately 75 per cent of the cases and permanent disability (as judged by the persistence of corneal haze) in nearly all cases.²¹⁴ In the rabbit eye a 0.1-mg dose of liquid L produces a maximal lesion. With doses greater than 0.1 mg the severity of the ocular reaction did not appreciably increase. It has been stated that a dose of 0.01 to 0.02 mg of liquid L will produce permanent ocular damage (in rabbits) approximately equal to that produced by 0.1 to 0.2 mg of liquid H. With 0.05 mg of L most of the eyes are completely destroyed, whereas even 1.4 mg of H does not produce an equally severe lesion. Mild, self-limiting injuries of comparable severity are produced by 0.005 mg of L and 0.02 mg of H. It is thus apparent that the severity of the L lesion increases steeply with increasing dosage and rapidly reaches a maximal lesion, whereas the curve relating severity of the lesion to dosage of H is much more flat and very large doses are required to destroy an eye completely.

The threshold Ct for vesication of bare human skin (forearm) has been estimated as 1.0 mg min/l (analytical) for a temperature of 55 F and relative humidity = 70 per cent.²⁵⁸ A Ct of 1.8 at $T = 90$ F and relative humidity = 49 per cent caused vesication of the bare hand in 50 per cent of the men exposed.²⁰⁹ A Ct of 1.5 (analytical) caused vesication of the neck of six men exposed in the field at $T = 66$ F and relative humidity = 41 per cent, but no effect was obtained on skin covered by ordinary battle dress.²⁵⁸ A Ct of 1.5 (analytical) at $T = 90$ F and relative humidity = 65 per cent caused vesication on the skin (forearm) of three men (3/3), whereas a Ct of 1.2 produced vesication in none of three men (0/3) under the same conditions of temperature and humidity.²¹⁹

Liquid L on the bare skin is a very potent vesicant, the median threshold blistering dose for man being 14 μ g as compared with 32 μ g for H.⁷⁸ Contrary to the opinions held in this country prior to World

War II, recent work has tended to establish the view³³⁹ that in relatively large amounts L does not produce as severe skin damage in man as does H. Although with doses up to about 1 mg of liquid L produces skin lesions in men not perceptibly different from those resulting from the same amount of liquid H, the response to larger doses of the two agents is different. For 2-mg dosages of L and of H, the lesions produced by L are less severe and heal in 2½ to 4 weeks compared to the 5 to 9 weeks required for healing of the mustard lesions. One investigation,³³⁹ using much larger doses, placed two large drops (from an ordinary eyedropper) of L on one forearm and of H on the opposite forearm of a man. He reported healing of the L lesions in 26 days and of the H lesions in 63 days and stated that these results were typical of other experiments. It has been pointed out that in rabbits the damage produced by 2 mg of liquid L is more severe and slower to heal than that produced by 2 mg of liquid H. The reaction of rabbit skin toward L is, therefore, not characteristic of the reaction of human skin. In an investigation conducted at Porton²⁵⁹ it was concluded that L burns heal more quickly than H burns, are less prone to infection, and cause less pain during healing. The question of the comparative severity of lesions produced by H and L on human skin has recently been reinvestigated,²²⁰ with the result that L lesions were found to be less severe and to heal more quickly than those caused by the same amount of H (either by weight or by volume, the dose being 1.0 mg or 0.5 microliters).

It may be noted parenthetically that in 1941 a statement appeared in United States official chemical warfare manuals to the effect that the fluid from lewisite bullae was itself vesicant. However, experiments have been reported³³⁹ leading to the conclusion that L blister fluid was neither vesicant nor irritating and an American investigation in 1943¹³⁷ confirmed this conclusion, with the result that statements regarding the vesicancy of L blister fluid have been withdrawn from recent editions of United States official manuals.

The toxicity of L for man is summarized in Table 5.

The dosages required for L to produce casualties in men or to kill them appear to have been as well established as would be possible through the use of experimental animals in lethal experiments and human observers in marginal experiments.

As was aptly stated in 1919²²⁵ the value of L as a military agent depends in large degree on whether

TABLE 5. Toxicity of lewisite for man.

	Vapor approx. $L(Ct)_{50}$ (analytical) mg min/l	Liquid dose mg ⁵⁰
Death (by inhalation)	1.2-1.5 (est.)	
Death (by body exposure)	100 (est.)	2,800 (est.)
Vesication of skin (bare)	1.2-1.5	0.014
Serious corneal damage	1.5 (est.)	0.1 (est.)

the necessary dosages can be set up in the field. Sufficient field experiments have now been carried out to indicate that the requisite dosages are probably not attainable with any reasonable expenditure of munitions.

FIELD TEST DATA

The concentration of vapor obtained from pouring 50-75 g of L per square yard on the ground is low and Ct values obtained are usually not over 4.0 mg min/l.^{124,250} The vapor concentration obtained directly over the contaminated area fell steeply during the first 30 minutes of the experiments and thereafter was not dangerous.

In experiments conducted at Edgewood Arsenal four M70 bombs charged L (total 360 pounds) were fired statically. Twenty-five yards downwind from the burst the initial concentration was 0.060 mg/l but fell to 0.013 mg/l in 10 minutes. The Ct for 15 minutes was 0.395 mg min/l.²¹⁶ In a further test at Edgewood an airplane sprayed 610 pounds of unthickened L from an altitude of 75 feet over an area of 76,250 square yards.¹⁶³ Significant vapor concentrations directly over the contaminated area were recorded only for the first 10 minutes and the total Ct recorded was of the order of 3.

It is apparent from the above examples that dangerous concentrations of L vapor are difficult to attain in the field. The reason for this is apparently the rapid hydrolysis of the vapor and liquid in contact with a moist environment, with possibly the destruction of some L by alkaline soil, together with the fact that the agent may be partially destroyed by detonation when loaded in munitions. In extremely hot and dry climates more effective vapor concentrations may be anticipated.

The effects of liquid L on bare skin might be achieved through ground contamination, bursting munitions, or airplane spray. However, L is so unstable on contact with moisture that under ordinary conditions of humidity it is rapidly hydrolyzed on the surface of soil or foliage, leaving behind a residue of L-oxide. The L-oxide, while weakly vesicant, is

nonvolatile and insoluble in water and is only effective when brought in contact with bare skin. If the soil is alkaline a part of both the original L and the oxide may be completely destroyed. The British²³⁹ attempted to assess the danger of systemic intoxication from liquid L released in bomb explosions. On the assumption that the lethal dose for man would be 1.9 g (a dose which is probably not fatal) it was concluded that the risk of receiving serious injury from a bomb charged with L would be no greater than from a bomb of the same size charged with high explosive. When unthickened L is released from an airplane spray tank, the droplets formed are less than 1 mm in diameter.¹⁶³ Since it has been reported^{229,230} that L droplets of less than 1 mm in diameter evaporate completely while falling through 2,000 feet, it is apparent that the employment of unthickened L from medium altitudes (>2,000 feet) as airplane spray would be useless. L may be thickened with methyl methacrylate and similar materials. The use of thickened L as airplane spray results in larger drops (55 per cent of drops ≥ 0.5 mg as compared with 8 per cent of drops ≥ 0.5 mg for unthickened L).¹⁶³ However, when droplets of thickened L strike a surface, they tend to harden. This effect may be due to the formation of a skin of L-oxide on the surface of the drop.¹⁶³

A comparison of the casualty-producing effect of thickened and unthickened L when used as an airplane spray from low altitude (100 feet) revealed that thickened L was less effective in producing casualties in goats than unthickened L, and that the eye damage caused by the unthickened L was more severe than that caused by thickened L.¹⁶³

The tactical value of producing L blisters on human skin is thrown into very serious doubt by recent Canadian experiments³¹³ in which observers clad in battle dress and shirts over long-limbed underwear and wearing respirators and steel helmets were exposed to airplane spray of L to which had been added 6.55 per cent of thickener. The temperature was 75 F with relative humidity = 39 per cent, and the contamination density was 0.7 to 5.4 g/m². The drops varied between 1.3 and 5.6 mm in diameter. Of 30 men hit by the spray, 20 developed lesions which in 7 cases were numerous and prominent but in other cases were trivial. It was noted that the individual lesions produced were discrete and circumscribed in contrast to the diffuseness of the typical lesion produced by H spray. After 9 days of comparatively strenuous exercise, none of the observers was

in such condition that he could not carry out military duties, and in no case had secondary infection developed. It was concluded that the casualty-producing propensities of H spray are definitely greater than those of L spray.

PROTECTION AGAINST LEWISITE

Lewisite Vapor. The median detectable concentration of L vapor by odor is stated to be 0.014 to 0.023 mg/l. However, the irritating effect of the gas on the eyes and respiratory passages is noticeable at far lower concentrations, variously estimated as 0.008 mg/l³²⁹ and as 0.006 mg/l.¹²⁴ On the basis of these figures, a concentration of 0.006 mg/l should certainly warn troops of the presence of gas and should lead to masking or to withdrawal from the toxic atmosphere. The service respirator gives entirely adequate protection to the eyes and respiratory tract against the effects of L vapor. Even in the absence of the respirator, serious eye effects from L vapor are unlikely to occur in conscious men since the immediate response of the eye to L vapor is lacrimation and blepharospasm, both of which protect against further exposure.

Ordinary clothing affords considerable protection against L vapor. It has been estimated²²⁴ that a single layer of dry cloth would protect against approximately three times the concentration of L that would produce a reaction on bare skin. The British²⁵⁸ estimated that a *Ct* of 3.0–4.0 mg min/l would be required to produce an effect under a single layer of dry serge. In another report, it was found²⁵⁷ that the penetration of cloth by L vapor decreases with increasing humidity, and it was suggested that the reason lies in reaction of L with moisture on the fibers of the cloth. Complete protection against L vapor was afforded by ordinary dungaree shirt material, S-330 ointment, and CC-2 impregnated cloth up to at least *Ct* 3.3 (analytical) under exposure conditions of 90 F and 65 per cent relative humidity with 4 hours wear of the clothing after exposure.²¹⁹

Wet clothing is much more effective in protecting against L vapor than dry clothing. It has been estimated²²⁴ that 100 times the concentration of L that would produce an effect on bare skin would be required to penetrate a single layer of wet cloth. In fact the British²⁵⁸ state that L vapor will not burn through wet clothing.

LIQUID LEWISITE

Liquid lewisite in the eyes is capable of causing severe damage. However, complete protection against

liquid L is afforded to the eyes by wearing the respirator or the eye shield or even by closing the eyes.

Liquid L will penetrate ordinary dry clothing, a drop of 2.5 mg (1.5 mm in diameter) generally causing vesication through dry service clothing in temperate climates.²³³ Under tropical conditions a 0.4-mg drop (0.77 mm in diameter) may produce vesication through light dry clothing.³²⁴

Wet clothing protects against liquid L by forming the insoluble and nonvolatile L-oxide before the agent can penetrate to the skin.³¹⁴ CC-2 impregnated clothing offers more protection against liquid L than does unimpregnated dry clothing, although 5.7 mg of L produced vesication through a single layer of CC-2 impregnated cloth,¹⁷⁹ indicating that the protection afforded against L is less than that against H.

COMPARISON WITH MUSTARD

The toxicity of L vapor and H vapor by inhalation are of the same order of magnitude. However, to produce systemic effects through the skin, eye damages, or skin vesication, significantly higher *Ct*'s are required for L than for H. Because of the rapid destruction of L liquid and vapor in contact with moisture or with an alkaline environment the requisite *Ct*'s for L would be extremely difficult to attain in the field. Further, L vapor, unlike H vapor, is not insidious but gives adequate warning of its presence by irritation of the eyes and respiratory passages.

Liquid L is more vesicant than liquid H but the burns from L do not incapacitate men to the same extent as do burns from H,³¹³ and the L burns heal more rapidly and are less painful than those from H. Liquid L on the skin or in the eyes produces an immediate stinging sensation which warns of its presence, whereas mustard is nonirritating at the time of application.

Mustard penetrates ordinary clothing much more readily than does L, and, since H is more stable than L, is a better choice both for terrain contamination and vapor return.

Mixtures of H and L have been suggested but have no advantage over H used alone except with respect to lower freezing point.

THERAPY

In 1941, the discovery of a powerful therapeutic agent against L and other arsenicals was announced.^{300f} This substance, 2,3-dimercaptopropanol-1, variously known by the code letters BAL and DTH, will not only destroy arsenicals on contact, but is capable of minimizing the damage from liquid

arsenicals in the eyes if applied from 1 to 10 minutes after exposure, and from liquid arsenicals on the skin if applied up to 1 hour after contamination.

A discussion of BAL is beyond the scope of this report except to say that an ointment containing BAL was available for issue to United States soldiers. This ointment was suitable for application to the skin or eyes and placed in the hands of the soldier a method of self-help for minimizing the effects of contamination from liquid arsenical agents. Preparations of BAL were available to physicians for parenteral administration and are effective in combatting systemic intoxication from arsenicals.

SUMMARY

By the end of the World War II, the toxicology of L had been worked out to the point where the dosages required to produce casualties or death in human beings were known with a degree of approximation that is probably sufficient for military purposes.

Field tests, however, showed little promise of attaining the requisite dosages of L vapor with any reasonable expenditure of munitions. The use of liquid L for gross contamination of personnel seems feasible only when the agent is dispersed as low-altitude airplane spray, and the effects produced on contaminated personnel are so inferior to those produced by mustard as to create strong prejudice against the use of L.

Since the powerful antiarsenical agent, BAL, available to Britain and the United States in World War II, will be available to all in the future, there seems to be little likelihood that there will ever be any incentive for the use of L as a chemical warfare agent.

7.3.2 Chlorarsine Derivatives Other Than Lewisite

LETHAL AGENTS

In the Spring of 1918, ethyldichlorarsine (ED) was used by the Germans as a skin and lung irritant suitable for gassing operations to be followed by infantry assaults.^{331,329} There is no mention in Allied official records of casualties attributed directly to ED, but the Germans held the compound in high regard. The United States Chemical Warfare Service investigated methyldichlorarsine (MD) during the latter half of 1918 but the compound was not used in battle.

In 1939, the results of a preliminary investigation by the Chemical Warfare Service¹²⁷ revealed a lack of sufficient data for making a definite decision as to

the value of ED as a military agent, but stated that "the present available data indicate sufficient potential value to warrant further study and development." Accordingly, the National Defense Research Committee [NDRC] was asked to screen the arsenicals for toxicity and stability in order to determine whether any members of the group were sufficiently promising to warrant further study or development as chemical warfare agents. A number of chlorarsine derivatives were prepared and were studied for toxicity at the University of Chicago Toxicity Laboratory [UCTL].

Physiological Action. The toxic chlorarsine derivatives produce effects which are qualitatively similar to those produced by L (q.v.) but which differ in degree. Thus, they are all irritant to the respiratory tract and produce lung injury on sufficient exposure. The vapors are irritating to the eyes and the liquids may produce serious eye lesions. The absorption of either vapor or liquid through the skin in adequate dosage may lead to systemic intoxication or death. Local skin damage leading to vesication in man is usually produced by sufficient exposure to the vapor or by contact with the liquid.

Vapor Toxicity. The chlorarsines originally screened for vapor toxicity at the UCTL²⁷ are listed in Table 6, which shows the results of tests against

TABLE 6. Toxicity of vapor of chlorarsines for mice. All figures for $L(Ct)_{50}$ are in mg min/l (nominal).

Compound	$L(Ct)$ — (Mouse)
Lewisite, isomer I	$L(Ct)_{50} = 2.8$
Lewisite, isomer II	$L(Ct)_{50} = 2.8$
Plant run lewisite, isomer I	
Phenyldichlorarsine	$L(Ct)_{50} = 3.7$
β -Chloroethyldichlorarsine	$L(Ct)_{90} \sim 13.$
β -Methoxyethyldichlorarsine	unstable
β -Ethoxyethyldichlorarsine	unstable
β -Chloromethoxypropyldichlorarsine	(No deaths at $Ct = 8.7$)
Allyl phenylchlorarsine	(No deaths at $Ct = 24.44$)
Phenyl(β -chlorovinyl)chlorarsine	$L(Ct)_{10} \sim 1.$
Isoamyldichlorarsine	$L(Ct)_{50} \sim 2.$
sec-Butyldichlorarsine	$L(Ct)_{50} \sim 12.$
bis(Chloromethyl)chlorarsine	$L(Ct)_{50} \sim 4.5$
Chloromethyldichlorarsine	(No deaths at $Ct = 43.5$)
4-Pentenylchlorarsine	$L(Ct)_{33} \sim 3.7$
Amyldichlorarsine	$L(Ct)_{50} = 2.5$
Butyldichlorarsine	$L(Ct)_{50} \sim 3.5$
Ethyldichlorarsine	$L(Ct)_{50} \sim 3.5$
β -Furyldichlorarsine	(No deaths at $Ct = 2.3$)
Heptyldichlorarsine	$L(Ct)_{80} \sim 13.1$
β -Methylbutyldichlorarsine	unstable
Hexyldichlorarsine	$L(Ct)_{50} \sim 3.$
Dimethylchlorarsine	$L(Ct)_{20} \sim 10.$

mice by total exposure for 10 minutes. On the basis of the information listed in Table 6 and information from the Chemical Warfare Service on ED,¹²⁹ butyldichlorarsine,¹³⁰ amyldichlorarsine,¹⁵⁷ and isoamyldichlorarsine,¹⁵⁷ a more detailed investigation was made of the toxicities toward mice (by total exposure) of the vapors of the alkyldichlorarsines from methyl- through hexyl-⁴³ In order to avoid errors known to result from different degrees of humidification of the animal's fur, the mice were exposed for 1 hour to a relative humidity of 20–30 per cent before exposure to the toxic arsenical. The dichlorarsines were vaporized with dry nitrogen at 25–30 C and were passed through the 4-l glass chamber at 11.2 lpm. The relative humidity of the gases in the chamber did not exceed 8 per cent.

The vapor toxicities of the alkyldichlorarsines are given in Table 7, together with the toxicity of phenyldichlorarsine (PD) and of L for purposes of comparison.

TABLE 7. Toxicity of vapor of dichlorarsines for mice.

All figures are $L(Ct)_{50}$ in mg min/l.

Exposure time = 10 min; observation period = 10 days.

Agent	Total exposure
Methyldichlorarsine	2.7 (anal.) ⁴³
Ethyldichlorarsine	1.555 (anal.) ⁴³
Ethyldichlorarsine	3.4 (nom.) ¹²⁹
Propyldichlorarsine	1.4 (anal.) ⁴³
Butyldichlorarsine	1.8 (anal.) ⁴³
Butyldichlorarsine	3.7 (nom.) ¹³⁰
Amyldichlorarsine	1.4 (anal.) ⁴³
Amyldichlorarsine	3.7 (nom.) ¹⁵⁷
Isoamyldichlorarsine	3.7 (nom.) ¹⁵⁷
Hexyldichlorarsine	1.5 (anal.) ⁴³
Phenyldichlorarsine	3.4 (nom.) ²⁷ ; 3.3 (nom.) ¹¹⁹
Lewisite	1.5 (anal.) ⁴³
Lewisite	2.8 (nom.) ²⁷

Examination of the data of Table 7 leads to the conclusion that all of the dichlorarsines tested, with the possible exception of MD, have essentially the same toxicity toward mice.

Fifty-three dihaloarsines were tested at the UCTL and the conclusion reached⁶⁸ that the members of the series vary in toxicity up to a maximum in the group that contains L, ED, and the homologous straight chain aliphatic dichlorarsines. Data have also been obtained for a number of monohalogenated arsines,⁶⁸ but none of these compounds are superior to L.

References to the vapor toxicities of other halogenated arsines will be found in Table 9.

The toxicity of several dichlorarsines when applied to the skin (shaved) of mice is shown in Table 8.

TABLE 8. Percutaneous toxicity of arsenicals for mice.²⁷

Compound	Dose mg	No. of mice	Per cent mortality 10-day period	Comparison with lewisite
L (plant run)	0.1	10	0	...
	0.3	10	50	...
	0.5	10	100	...
ED	0.1	4	0	...
	0.5	4	25	< $\frac{1}{3}$ L
	1.0	4	25	...
N-Butyldichlorarsine	0.5	10	10	...
	1.0	10	30	< $\frac{1}{3}$ L
β -Methylbutyldichlorarsine	0.5	4	25	...
	1.0	4	100	$\sim \frac{1}{2}$ L
N-Amyldichlorarsine	0.1	7	14	...
	0.3	10	80	= L
	0.5	10	100	...
Hexyldichlorarsine	0.1	4	0	...
	0.5	4	50	...
	1.0	4	50	$\sim \frac{1}{2}$ L
Heptyldichlorarsine	0.1	4	0	...
	0.5	4	0	$\sim \frac{1}{3}$ L
	1.0	4	50	...
PD	0.1	10	20	...
	0.3	10	30	= L
	0.5	10	100	...

These data show that none of the dichlorarsines tested are more toxic than L and that only amyldichlorarsine and PD equal L in systemic toxicity.

Thirty-five dihalogenated arsines and thirteen monohalogenated arsines were examined for vesicancy at the UCTL⁶⁸ without revealing any vesicant superior to lewisite.

In general, the dichlorarsines are better vesicants than the monochlorarsines,⁷⁹ and the simple alkyldichlorarsines compare favorably with L in respect to "absolute" vesicancy, i.e., when evaporation of the liquid from the skin is prevented by covering.

The introduction of a single chlorine atom on the terminal carbon of a normal aliphatic substituent in a dichlorarsine or the use of branched chain substituent groups results in loss of vesicant potency.²⁷ Thus, ED is a more potent vesicant than L²⁷ when evaporation from the skin is prevented, and amyldichlorarsine is a better vesicant than isoamyldichlorarsine.^{27,157}

EYE EFFECTS

The vapors of the chlorarsines are generally irritating to the eyes, leading to lacrimation and bleph-

arospasm which protect against further damage. Liquid MD produces a lesion in the rabbit eye which is less severe than one caused by L.^{299c} Liquid ED produces a lesion in the rabbit eye which is comparable in severity to that caused by L.^{299b} BAL is effective in the prevention of eye damage from either MD or ED.^{299b,c}

Assessment of the Military Value of Chlorarsines Other than L. Of all the chlorarsines studied, only MD, ED, PD, butyldichlorarsine, and the amyldichlorarsines approach L in toxicity and vesicant potency. Of these, butyldichlorarsine is too unstable¹³⁰ and the amyldichlorarsines too difficult to prepare¹⁵⁷ to be considered as chemical warfare agents.

Thus, after an exhaustive examination of many compounds, it appears that the best of the chlorarsines other than L are those which were used (ED and PD) or considered for use (MD) in World War I. The status of MD, ED, and PD as military agents has recently been reviewed²¹⁶ with the following results:

1. MD. The vapor is so irritating that it is easily detected at low concentrations and would lead to prompt masking. The vapor is easily hydrolyzed and the dosage required for skin vesicancy so high that there is no hope of obtaining vesicant dosages of vapor in the field. The skin and eye effects of the liquid are not so damaging as those produced by L.

2. ED. Ethyldichlorarsine is somewhat superior to MD but is inferior to L as a casualty agent.

3. PD. The vesicancy, systemic toxicity, and toxicity by inhalation of PD are equal to those of L, but PD penetrates clothing less effectively than L and the volatility of PD is so low that casualties from exposure to the vapor are hardly to be expected in the field. Like MD and ED, PD is easily hydrolyzed.

In view of these facts, it appears that the best of the chlorarsines are inferior to L and, since L itself does not appear to have any future as a chemical warfare agent, it can be assumed that the other chlorarsines will not be considered further as military agents for casualty effect. It is interesting to note, however, that the Allies captured a considerable number of German artillery shells charged with a mixture of mustard and PD. Whether this indicates that the Germans held a higher opinion of the effectiveness of PD than the Allies or the mixture was dictated by other considerations is not clear at the present time.

7.3.3 Arsine and Nonhalogenated Arsine Derivatives

ARSINE

During World War I, the Allies did considerable exploratory work on the potentialities of arsine as a chemical warfare agent. In 1919 it was stated:¹²⁵

During the war many suggestions were made that arsine should be used. The popular plan was to use magnesium arsenide which would hydrolyze in moist air, setting free arsine. The experiments made by the Research Division showed that the hydrolysis does not take place rapidly enough under ordinary conditions to give an efficient concentration of arsine. At the time of the armistice experiments were still under way to determine whether this material could be used effectively in the rain. While the use of magnesium arsenide or of any arsenide was not very promising, there seemed to be a distinct possibility of using liquid arsine . . . If arsine is to be used in warfare, it seems probable that it must be used as liquid.

In 1939, the available data concerning arsine as a potential chemical warfare agent were summarized¹²⁵ with the conclusion that its value would depend on whether the canister of the gas mask would afford sufficient protection against it under all conditions to which the canister might be exposed.

It was recognized that arsine might be useful as a casualty agent aside from its lethal effects and accordingly studies of the toxicity and suitability of the compound for chemical warfare use were reinvestigated by both the Americans and the British.

Physiological Action. The physiological action of arsine has been well summarized in the open literature.³³⁰

In vitro studies have shown that arsine is oxidized aerobically in aqueous solution, and that this oxidation is catalyzed by hemoglobin.^{294a} In the presence of arsine and oxygen, however, the hemoglobin undergoes destruction forming a number of compounds including methemoglobin, and a tetrapyrrolic compound whose spectrum resembles that of sulfmethemoglobin.^{294b,304d,h} During the reaction of arsine with hemoglobin about 40 per cent of the arsine taken up is held in a nondialyzable form, while the remainder is mostly arsenite with a small amount of arsenate. There is no reaction between arsine and hemoglobin under strictly anaerobic conditions.^{294b}

Arsine is a strong hemolytic agent *in vivo*; and *in vitro* under aerobic conditions only.^{294b} In view of the known oxidation products of arsine, experiments were carried out to determine whether the hemolytic effects of arsine were due to arsenite or arsenate rather than to arsine *per se*, but with negative results.⁹³

The action of arsine on tissue slices has been studied and compared with that of arsenite, with the conclusion that the effect of arsine in reducing the oxygen uptake of kidney slices is similar to that of arsenite.^{93,304c} BAL protects kidney slices against the effects of arsine but not of arsenite.⁹³ The action of arsine on liver slices is not identical with that of arsenite since the toxicity of arsine increases more rapidly with increasing concentration, and liver slices treated with arsine change color, suggesting a reaction with heme compounds that does not occur with arsenite-treated liver slices.^{304c}

Toxicity. Available data on the toxicity of arsine by inhalation have been summarized.²¹⁶ The data cited are quite variable both for exposures of a given species and for different species. The LC_{50} for mice has been determined as of the order of 0.250 mg/l for a 10-minute exposure;^{121,216,304f} but studies at the UCTL²³ resulted in a figure of 0.520 ± 0.100 mg/l (analytical), with no apparent explanation of the discrepancy.

There do not appear to be any satisfactory data for the LC_{50} for dogs with 10-minute exposure, but 0.35 mg/l for a 30-minute exposure is said to be the LC_{50} ,¹⁵⁸ and lethal concentrations for various exposure periods have been compiled.²¹⁶ Rabbits are apparently less susceptible to arsine than mice, the LC_{50} for 10-minute exposure being estimated to lie between 0.65 and 0.96 mg/l.¹⁷⁸ No satisfactory LC_{50} has been reported for cats, but 0.80 mg/l for 10 minutes caused the death of 3/4 cats within 18 hours (G-2 Report No. 1322²¹⁶), whereas cats exposed to 4.1 mg/l for 1 minute did not die.²³

The LC_{50} for rats on 10-minute exposure is of the same order as that for mice, being between 0.39 and 0.66 mg/l (G-2 Report No. 1322²¹⁶). The LC_{50} for goats on 10-minute exposure is estimated as being between 1.0 and 2.2 mg/l (G-2 Report No. 1322²¹⁶). Four of five monkeys died after exposure to 0.45 mg/l for 15 minutes.^{304f}

No data exist for the LC_{50} for man, but the minimum disabling concentration has been estimated as 2.0 mg/l for 2 minutes or 0.2 mg/l for 30 minutes.¹⁵⁸ Henderson and Haggard state that exposure to a concentration of arsine between 0.051 and 0.191 mg/l would be dangerous after 30 minutes, whereas exposure to 0.798 mg/l would be fatal after 30 minutes.³²⁸ British estimates based on the assumption that 2 mg/kg of arsine would be fatal to man put the casualty-producing Ct at 14 mg min/l for a man at rest and at 4.66 mg min/l for a man working; and the

fatal Ct at 28 mg min/l and 93 mg min/l for a resting man and working man respectively.³¹¹ Early British results indicated that for the effect of arsine on mice the product C^2t rather than Ct was a constant, but later investigation showed that for concentrations greater than 0.5 mg/l, Ct was constant, whereas for concentrations less than 0.5 mg/l, C^2t was constant.^{304f}

On the grounds that the incapacitation of troops may be as valuable as their death in most military situations, and that the incapacitating dose of an agent may be quite different from the lethal dose, studies were carried out on rabbits to examine the possibilities.¹⁷⁸ The results indicated that exposure of rabbits to 0.05 mg/l for 10 minutes caused significant changes in the oxygen-carrying capacity of their blood, but that the effect was transient. After 10-minute exposure to concentrations between 0.13 and 0.20 mg/l the rabbits were no longer able to maintain a relatively high red blood cell count, and the decrease in oxygen-carrying capacity of the blood was severe in about half of the animals, whereas with 10-minute exposures to concentrations between 0.234 and 0.40 mg/l a marked decrease in hemoglobin was invariably noted. A similar decrease in the hemoglobin content of human blood might be expected to cause severe but sublethal casualties.

Therapy. Dithiol compounds are effective in the treatment of arsine poisoning, although BAL-ethyl ether (2,3-dimercaptopropyl ethyl ether) is more effective than BAL itself.^{98g} Since BAL-ethyl ether is tolerated by human beings in therapeutic dosages without toxic symptoms, the compound appears to be suitable for the treatment of arsine poisoning in man.²¹⁶

Assessment of Value as a Chemical Warfare Agent. The conclusion of the United States Chemical Warfare Service in 1939 was that the value of arsine as a chemical warfare agent would depend on the question of canister protection.¹²⁵ The British in 1941 concluded that the only potential method for the liberation of arsine would be by high-capacity bombs and that the only possible advantage over gases of the phosgene type would be that its detection at low concentration is more difficult. In order to utilize low concentrations of arsine, however, exposure must be prolonged and this is very difficult to obtain short of excessive effort, so that on the whole arsine should not merit any particular consideration as an offensive weapon, provided respirator protection is adequate.²³⁶

The question of canister protection against arsine

has been summarized as follows: "At one time arsine was thought to be a very promising war gas because it penetrates humidified unimpregnated or copper oxide impregnated charcoal very readily. With the introduction of silver impregnation, however, the protection against arsine was made almost comparable to phosgene. . . ." ¹⁰⁰

The weight of arsine that would have to be expended to produce a lethal concentration is theoretically about 10 times as great as the weight of phos-

gene required for the same purpose.²³⁶ Since, in addition, modern respirators give adequate protection against it, arsine shows little promise in chemical warfare.

NONHALOGENATED ARSINE DERIVATIVES

A number of tertiary arsine derivatives have been examined for toxicity. Data for 51 such compounds were obtained by the UCTL,⁶⁸ and reference to these and to other tertiary arsines are listed in Table 9.

TABLE 9. Arsenical compounds examined as candidate chemical warfare agents.

The compounds in Table 9 are arranged in the following categories:

1. Derivatives of arsine.
2. Derivatives of primary arsines.
3. Derivatives of secondary arsines.
4. Tertiary arsines.
5. Quaternary arsenic derivatives.
6. Arsenic analogs of hydrazine.
7. Derivatives of arsenic oxides, sulfides, and amines.
8. Halogen and oxygen derivatives of tertiary arsines.
9. Derivatives of arsenic, arsonic, and arsinic acids.
10. Arsenic derivatives of uncertain constitution.

British reports describing the examination of compounds marked with an asterisk are not all available.

Centigrade scale is used throughout the table.

Compound	Reference to synthesis	Physical properties		Reference to toxicity data	
		Property	Reference		
<i>Derivatives of arsine</i>					
1. Calcium arsenide	311	d^{15}	2.5	311	311
2. Arsine	296b, 311	d^0	1.44	296b	23, 311
	...	mp	116.1–116.0°	296b	...
	...	bp	62.8°	296b	...
3. Arsenic trifluoride	311, 333	d^4	2.6659	298a	68, 311
	...	mp	8.5°	298a	...
	...	bp	60.4°	298a	...
	...	vol	152	311	...
4. Arsenic trichloride*	...	$n_D^{14.5}$	1.6009	311	252
	...	d^{20}	2.163	244	...
	...	mp	13°	311	...
	...	bp ⁷⁶⁰	129–130°	244	...
	...	vol ²⁰	84	311	...
5. Arsenic trichloride — dioxane complex*	...	...		...	227
6. Arsenic trichloride — thioxane complex*	...	...		...	227
7. Arsenic pentafluoride	342	mp	80.4°	298a	...
	...	bp	52.8°	298a	...
<i>Derivatives of primary arsines</i>					
8. Methylarsine	5	bp	2°	5	...
9. Methyl difluorarsine*	296c, 298a	d	1.9725	298a	298a
	...	mp	30°	296c, 298a	...
	...	bp	76°	296c, 298a	...
10. Methyl dichlorarsine*	32, 290j, 311	n_D^{29}	1.5588	32	27, 43, 68, 311
	...	d^{20}	1.8358	32	...
	...	mp	42.5°	32	...
	...	bp ⁷⁶⁰	132.5°	32	...
	...	vol ²⁰	74.4	311, 70	...
	...	...	68.3	...	...
11. Chloromethyl dichlorarsine	47	bp ¹⁰	53°	47	27, 68
	...	vol	135	27	...

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference to toxicity data
		Property	Reference	
12. 2-Chlorovinyl difluoroarsine*	113, 180, 296c	d^{25}	1.97	180
	...	mp	26°	180
	...	bp ^{14.5}	43.5°	296c
	...	bp	105–110°	180
	...	vol ²⁵	31.77	180
13. 2-Chlorovinyl dichloroarsine* Lewisite (isomer 1)	See Bibliography	n_D^{25}	1.6073	43
	...	d^{25}	1.879	27
	...	mp	2.4°	27
	...	bp ¹⁰	75°	43
	...	bp ⁷⁶⁰	190°	27
	...	vol ²⁰	2.3	311
	...	vol ²⁰	4.47	70
Lewisite (isomer 2)	See Bibliography	n_D^{25}	1.5900	27
	...	d^{25}	1.8681	27
	...	bp ¹⁰	62.8°	27
	...	bp ⁷⁶⁰	150.2°	27
14. 2-Chlorovinyl dichloroarsine-dioxane complex*	...	...	...	227
15. 2-Chlorovinyl dibromoarsine*	231	bp ¹⁷⁻¹⁸	106–107°	231
16. 2-Bromovinyl dibromoarsine*	231	bp ¹⁸	132–137°	231
17. 2,2-Dichlorovinyl dichloroarsine*	311	...	...	311
18. Ethylarsine	39	d^{26}	1.217	39
	...	bp ⁷³⁵	35–36°	39
19. Ethyl difluoroarsine*	112, 296c	d	1.743	296c
	...	mp	38.7°	296c
	...	bp	94.3°	296c
20. Ethyl dichloroarsine* (ED)	5, 32, 48, 58, 311	$n_D^{14.5}$	1.5588	311
	...	d^{20}	1.6595	32
	...	bp ⁷³⁵	153–155°	5
	...	vol ²⁰	21.9	127
	...	vol ²⁰	30.2	70
21. Ethyl dibromoarsine	58	n_D^{26}	1.6405	58
	...	d^{27}	2.403	58
	...	bp ¹⁶	87–88°	58
	...	vol ²⁰	5.72	70
22. 2-Chloroethyl dichloroarsine*	1, 111, 114	bp ¹⁸	99.8–100°	1
23. 2-Hydroxyethyl dichloroarsine*	...	...	...	227
24. 2-Methoxyethyl dichloroarsine	1	d^{20}	1.693	1
	...	bp ⁶	94–95°	1
	...	bp ¹⁵	102–103°	1
25. 2-Ethoxyethyl dichloroarsine	1	d^{20}	1.605	1
	...	bp ¹⁰	95–97°	1
26. Allyl dichloroarsine	1, 32, 58, 105	n_D^{20}	1.5702	1
	...	d^{27}	1.6294	32
	...	bp ^{4.5}	42°	32
27. 3-Chlorallyl dichloroarsine	301c	bp ¹⁸	104–105°	301c
28. Propyl dichloroarsine*	32	n_D^{28}	1.5297	32
	...	d^{20}	1.5380	32
	...	mp	28.2°	32
	...	bp ⁷⁵	99°	32
	...	bp ⁷⁶⁰	175.3°	32
	...	vol ²⁰	12.4	70
29. Propyl dibromoarsine*	...	...	...	227
30. Propyl dicyanoarsine	39	mp	82–86°	39
31. Isopropyl dichloroarsine*	...	...	...	227
32. 3-Chloropropyl dichloroarsine*	311	...	...	311
33. 3-Chloromethoxypropyl dichloroarsine	5	bp ⁵	136–137°	5

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
34. 2-Chloro-3-(2-chloroethylthio)-1-butenyldichlorarsine*	...	...	...	...	227
35. Butyldichlorarsine*	1, 32, 130	d^{20}	1.4664	32	27, 43, 79, 121, 130
	...	bp ⁷⁶⁰	194°	32	...
	...	vol ²⁵	6.3	130	...
36. Butyldibromoarsine*	...	...	...	...	227
37. Butyldicyanoarsine	58	mp	61–63°	58	68, 79
38. <i>sec</i> -Butyldichlorarsine*	32	n_D^{23}	1.5245	32	27, 68, 79
	...	d^{20}	1.4128	32	...
	...	bp ⁷⁶⁰	182°	32	...
	...	bp ^{1.3}	39°	32	...
39. 2(or 4)-Chloro-3-methyl-1,3 (or 1,2)-butadienyl- dichlorarsine*	...	...	...	...	227
40. 4-Pentenyl-dichlorarsine	32	n_D^{26}	1.5698	32	27, 68, 79
	...	d^{26}	1.453	32	...
	...	bp ^{3.5}	102.5–105.5°	32	...
	...	bp ²⁶	111–114°	32	...
	...	vol	0.12	27	...
41. 2-Chloro-1-pentenyl-dichlorarsine	38	bp ²⁶	130–133°	38	68, 79
42. Amylarsine	58	bp ⁷³⁰	125–127°	58	68
43. Amyldichlorarsine*	28, 32, 58	n_D^{25}	1.5177	32	27, 43, 157, 79
	...	d^{20}	1.4035	32	...
	...	bp ³⁰	118°	32	...
	...	bp ⁷⁶⁰	213°	32	...
44. Amlydibromoarsine	39	$n_D^{31.5}$	1.5760	39	68, 79
	...	d^{30}	1.8804	39	...
	...	bp ¹⁸	125.5–127°	39	...
	...	bp ⁷³⁴	248°	39	...
	...	vol	0.399	70	...
45. Amyldicyanoarsine	58	mp	69–69.5°	58	68, 79
46. Isoamyldichlorarsine	28, 32	n_D^{25}	1.5157	32	68, 79, 157
	...	d^{28}	1.3904	32	...
	...	bp ⁴	72.5–74°	32	...
47. 2-Methylbutyldichlorarsine	32	n_D^{26}	1.5183	32	27
	...	d^{26}	1.4302	32	...
	...	bp ²¹	101–105°	32	...
48. Hexyldichlorarsine	32	n_D^{26}	1.5122	32	43, 68, 79
	...	d^{27}	1.352	32	...
	...	bp ²⁸	125–127°	32	...
49. Hexyldicyanoarsine	58	mp	67.8–69.8°	58	68
50. Heptyldichlorarsine	32, 58	n_D^{27}	1.5102	32	27, 68, 79
	...	d^{27}	1.3206	32	...
	...	bp ¹⁴	130–131.5°	32	...
51. Phenylarsine	58	n_D^{27}	1.5967	58	68, 79
	...	d^{25}	1.524	58	...
	...	bp ⁶⁵	85–88°	58	...
	...	bp ⁵²	80–83°	58	...
52. Phenyl-difluoroarsine*	109, 296c	mp	42°	296c	121, 252
53. Phenyl-dichlorarsine (PD)*	58, 311	n_D^{25}	1.6332	58	27, 79, 252 311
	...	d^{25}	1.650	58	...
	...	bp ²³	137–140°	58	...
	...	bp	252–254°	58	...
	...	vol ²⁰	0.404	311	...
	...	vol ²⁰	0.280	70	...
54. Phenyl-dibromoarsine*	...	...	...	...	252
55. Phenyl-diiodoarsine*	...	...	...	...	252
56. <i>o</i> -Chlorophenyl-dichlorarsine*	58, 301e	n_D^{31}	1.6380	58	68, 79
	...	d^{31}	1.747	58	...
	...	mp	44–45°	58	...
	...	bp ²⁵	163.5–165°	58	...

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference to toxicity data
		Property	Reference	
57. <i>m</i> -Chlorophenyldichlorarsine*	311	mp	4°	311
	...	bp ⁸	124°	311
	...	bp ¹⁸	154°	311
58. <i>p</i> -Chlorophenyldichlorarsine*	311	...		311
59. <i>o</i> -Bromophenyldichlorarsine*	...	...		227
60. <i>m</i> -Bromophenyldichlorarsine*	311	mp	8–10°	311
	...	bp ¹¹	162°	311
61. <i>p</i> -Bromophenyldichlorarsine*	311	mp	11.8°	311
	...	bp ⁸	156°	311
62. <i>o</i> -Nitrophenyldichlorarsine*	58, 311	mp	49°	58, 49, 79, 311
63. <i>m</i> -Nitrophenyldichlorarsine*	5, 58, 311	mp	55°	5, 49, 68, 79, 311
64. <i>p</i> -Nitrophenyldichlorarsine*	5	mp	54–55°	5, 49, 79
	...	bp ²	170°	5, ...
65. 2,4-Dinitrophenyldichlorarsine	58	mp	69–70.5°	58, 49, 79
66. <i>o</i> -Hydroxyphenyldichlorarsine*	311	mp	77°	311, 311
67. <i>o</i> -Methoxyphenyldichlorarsine*	...	...		227
68. <i>p</i> -Methoxyphenyldichlorarsine*	...	...		227
69. 3,5-Dinitro-4-ethoxyphenyldichlorarsine	58	mp	81–82.3°	58, 68, 79
70. <i>p</i> -2-Chloroethylthiophenyldichlorarsine*	5	bp ^{0.004}	150°	283, 283, 291g
	...	bp ^{0.25}	186–193°	5, ...
71. 2-Phenoxyphenyldichlorarsine*	...	...		227
72. 2-(2'-Chlorophenoxy)phenyldichlorarsine*	...	...		227
73. 2-(3'-Chlorophenoxy)phenyldichlorarsine*	...	...		227
74. 2-(4'-Chlorophenoxy)phenyldichlorarsine*	...	...		227
75. <i>m</i> -N-Chloroacetylaminophenyldichlorarsine*	307q	mp	105°	307q, 227
76. N-Acetyl-N-phenyl- <i>p</i> -aminophenyldichlorarsine*	...	...		227
77. <i>p</i> -Tolylarsine		...		68, 79
78. <i>o</i> -Tolyldichlorarsine*	...	...		227
79. 2-Methyl-6-nitrophenyldibromoarsine*	...	...		227
80. <i>m</i> -Trifluoromethylphenyldichlorarsine	84h	...		68
81. <i>p</i> -Tolyldichlorarsine*		...		227
82. 2-Chloro-4-methylphenyldichlorarsine*	...	...		227
83. 2-Bromo-4-methylphenyldichlorarsine*	...	...		227
84. 3,5-Dinitro-4-methylphenyldichlorarsine	58	mp	126–127.5°	58, 68, 79
85. <i>o</i> -Dichlorarsinobenzoyl chloride*	...	...		227
86. <i>o</i> -Dichlorarsinobenzoic acid*	58	mp	159–169°	58, 68, 79
87. Benzyldichlorarsine	311	bp ⁵⁰	175°	311, 311
88. <i>p</i> -Acetylphenyldichlorarsine*	...	...		227
89. <i>m</i> -Chloroacetylphenyldichlorarsine*	5	bp ¹⁵	215–218°	5, 227
90. <i>p</i> -Chloroacetylphenyldichlorarsine*	...	...		227
91. <i>p</i> -Xenyldichlorarsine*	...	...		227
92. 2-(4-Chloroacetylphenyl)phenyldichlorarsine*	...	...		227
93. 2-Fluorenedichlorarsine*	...	...		227
94. 2-Fluorenonedichlorarsine*	...	...		227
95. <i>o</i> -Benzoylphenyldichlorarsine*	...	...		227
96. 2,2'-bis(Dichloroarsino)stilbene*	...	...		227
97. 2-Naphthyldichlorarsine	73	mp	74.6–75.1°	73, 68, 79
98. 2-Furyldichlorarsine*	1, 25, 301d, 311	<i>n</i> _D ^{29.5}	1.6092	25, 27, 311
	...	<i>d</i> ³⁰	1.797	25, ...
	...	bp ^{1.2}	85–98°	25, ...
99. 2-Thienyldichlorarsine*	311	bp ¹¹	118–122°	311, 311
100. 3-Pyridyldichlorarsine hydrochloride*	39	mp	229–235°	39, 68
101. Quinolyl-8-dichlorarsine	84h	...		...
102. 8-Methylquinolyl-5-dichlorarsine hydrochloride	84g	mp	181–182°	101d, 68
103. 2-(2-Picolyl)phenyldichlorarsine*	...	...		227
104. 2-(2-Picolyl)phenyldichlorarsine monohydrate*	...	...		227
105. 2-(2-Picolyl)phenyldichlorarsine hydrochloride*	...	...		227
106. 2-Dichlorarsinodibenzothiophene	84g	mp	114–115°	101d, 68, 79
107. N-Ethyl-3-dichlorarsinocarbazole	84e	...		...
108. 2-Dichlorarsinodibenzo- <i>p</i> -dioxin	84g	mp	108–109°	101d, 68, 79

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
109. 2-Dichlorarsinophenoxthiin	84g	mp	64–65°	101d	68, 79
110. 6-Dichlorarsino-2-phenylbenzthiazole hydrochloride	84h	...		...	...
111. <i>bis</i> (2-Dichlorarsinoethyl)sulfone	1	mp	79.5–80.5°	1	68
112. <i>o</i> -Phenylene- <i>bis</i> (dichlorarsine)*	...	...		...	227
113. <i>m</i> -Phenylene- <i>bis</i> (dichlorarsine)*	...	...		...	227
114. <i>p</i> -Phenylene- <i>bis</i> (dichlorarsine)*	58, 311	<i>d</i>	2.15	311	68, 79, 311
	...	mp	97–98°	58	...
	...	bp ²⁰	200°	311	...
115. 1,4- <i>bis</i> (Dichlorarsino)-2-nitrobenzene	58	mp	72.6–74.3°	58	68, 79
116. <i>p</i> -Dichlorostibinophenyldichlorarsine*	...	...		...	227
117. 2-Dichlorarsinodiphenylchlorarsine*	...	...		...	227
118. <i>bis</i> (<i>p</i> -Dichlorarsinophenyl) disulfide*	307l	mp	125.5–126.5°	307l	283, 291g
119. 3,3'- <i>bis</i> (Dichlorarsino)azoxybenzene	58	mp	119–120°	58	68
120. 4,4'- <i>bis</i> (Dichlorarsino)biphenyl*	...	...		...	227
<i>Derivatives of secondary arsines</i>					
121. Dimethylfluorarsine*	...	...		...	227
122. Dimethylchlorarsine (cacodyl chloride)	8, 38, 140, 131	<i>d</i> ²⁵	1.5	38	27, 131
	...	<i>d</i> ²⁵	1.489	145	...
	...	bp	103–105	38	...
	...	vol ²⁵	254.5	131	...
123. Dimethylecyanoarsine (cacodyl cyanide)	58, 317	<i>n</i> _D ^{39.5}	1.4859	58	68, 79, 151
	...	mp	35.3–35.8°	58	...
	...	bp	160–161°	58	...
124. Dimethylthiocyanoarsine	73	<i>n</i> _D ²⁹	1.6100	73	68, 79
	...	<i>d</i> ²⁵	1.4695	73	...
	...	bp ²⁵	106–107°	73	...
125. <i>bis</i> (Chloromethyl)chloroarsine	47	<i>d</i>	ca. 1.85	27	27, 68, 79
	...	bp ¹⁰	75°	47	...
126. Reaction product of mercury chloroacetylde and arsenic trichloride*	...	...		...	227
127. <i>bis</i> (2-Chlorovinyl)chlorarsine* (lewisite II)	311	<i>n</i> _D ¹¹	1.6096	311	235, 311
	...	<i>d</i> ¹¹	1.7047	311	...
	...	bp ¹⁰	112°	311	...
	...	bp ³⁰	136°	311	...
128. <i>bis</i> (2-Chlorovinyl)cyanoarsine*	...	...		...	227
129. <i>bis</i> (2,2-Dichlorovinyl)chloroarsine*	311	...		...	311
130. <i>bis</i> (2-Bromovinyl)bromoarsine*	231	bp ¹⁵	153–158°	231	231
131. <i>bis</i> (1,2,2-Trichlorovinyl)chloroarsine*	231	bp ⁵	141°	231	231
132. Diethylchlorarsine	58	<i>n</i> _D ²⁷	1.5080	58	68, 79
	...	<i>d</i> ²⁵	1.368	58	...
	...	bp ¹³	52–54°	58	...
	...	bp ⁷³⁶	156°	58	...
133. Diethylecyanoarsine	58	<i>n</i> _D ²⁷	1.4863	58	68, 79
	...	<i>d</i> ²⁵	1.238	58	...
	...	bp ¹³	80–81°	58	...
	...	bp ⁷³⁷	190–191°	58	...
134. Ethylpropylchlorarsine	39	<i>d</i> ³⁰	1.330	39	68, 79
	...	bp ³¹	82–85°	39	...
	...	bp ⁷²⁹	176°	39	...
135. Ethylpropylecyanoarsine	58	<i>n</i> _D ²⁷	1.4838	58	68, 79
	...	<i>d</i> ²⁵	1.194	58	...
	...	bp ²⁷	110–113°	58	...
	...	vol ²⁰	1.45	70	...
136. Ethylpropylthiocyanoarsine	73	<i>n</i> _D ²⁹	1.5674	73	68, 79
	...	<i>d</i> ²⁹	1.286	73	...
	...	bp ^{0.65}	102–110°	73	...
137. Ethylbutylchlorarsine	58	<i>n</i> _D ²⁶	1.5025	58	68, 79
	...	<i>d</i> ²⁶	1.272	58	...
	...	bp ¹⁹	89–92°	58	...

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
138. Ethylbutylecyanoarsine	58	n_D^{27}	1.4828	58	68, 79
	...	d^{27}	1.152	58	...
	...	bp ¹⁹	112–112.5°	58	...
139. <i>bis</i> (2-Chloro-3-(2-chloroethylthio)-1-butenyl) chlorarsine*	...	...		...	227
140. Dibutylidoarsine	84b	...		...	68, 79
141. <i>bis</i> (Cyclohexyl)chlorarsine*	...	...		...	227
142. Methylphenylarsine	73	d^{23}	1.31	73	...
	...	bp ³⁷	108–111°	73	...
	...	bp ⁴¹	128–129°	73	...
143. Methylphenylchlorarsine	58	n_D^{31}	1.6022	58	68, 79
	...	d^{31}	1.449	58	...
	...	bp ²³	127°	58	...
144. Methylphenyliodoarsine*	...	...		...	...
145. Methylphenylecyanoarsine	58	n_D^{26}	1.5812	58	68, 79
	...	d^{25}	1.372	58	...
	...	bp ²⁰	147–148°	58	...
146. Methylphenylthiocyanoarsine	58	n_D^{31}	1.6577	58	68, 79
	...	d^{32}	1.433	58	...
	...	bp ^{15–18}	176–179°	58	...
147. <i>m</i> -Chlorophenylmethylchlorarsine*	...	...		...	227
148. Methyl- <i>m</i> -nitrophenylchlorarsine	73	n_D^{30}	1.6272	73	68, 79
	...	d^{32}	1.617	73	...
	...	bp ^{0.8}	162°	73	...
149. Methyl- <i>m</i> -nitrophenylbromoarsine	73	n_D^{34}	1.6551	73	79
	...	d^{32}	1.857	73	...
	...	bp ^{0.85}	174°	73	...
150. Methyl- <i>m</i> -nitrophenylecyanoarsine	73	mp	79.5–80.5°	73	68, 79
151. Methyl-2-phenoxyphenylchlorarsine*	...	...		...	227
152. 2-Chlorovinylphenylchlorarsine	1, 231	d^{23}	1.401	27	68, 79, 231
	...	bp ⁸	138–145°	231	...
	...	bp ⁴	165°	27	...
153. <i>m</i> -Chlorophenyl-2-chlorovinylchlorarsine*	...	...		...	227
154. <i>m</i> -Chlorophenyl-2-chlorovinylecyanoarsine*	...	...		...	227
155. <i>o</i> -Carboxyphenylmethylchlorarsine*	...	...		...	227
156. α -(Phenylchlorarsino) acetic acid*	...	...		...	227
157. Allylphenylchlorarsine	1	d	1.479	1	27, 68, 79
	...	bp ⁵	99.5–100°	1	...
158. Methylphenethylchlorarsine*	...	...		...	227
159. 2-Chlorovinyl- α -furylchlorarsine	267	bp ¹	90–103°	267	267
160. 2-Chlorovinyl- α -furylecyanoarsine	267	bp ⁵	127–128°	267	267
161. Ethylene- <i>bis</i> (phenylchlorarsine)*	...	...	...	...	227
162. Diphenylfluoroarsine*	...	...		...	227
163. Diphenylchlorarsine (DA)*	73, 311	n_D^{33}	1.6429	73	79, 68, 311, 249
	...	d^{32}	1.413	73	...
	...	mp	38°	244	...
	...	bp ^{0.7}	157–160°	73	...
	...	vol ²⁰	<0.0001	311	...
164. 2-Chlorophenylphenylchlorarsine*	301e	mp	30–35°	301e	227
165. 3-Chlorophenylphenylchlorarsine*	...	...		...	227
166. 4-Chlorophenylphenylchlorarsine*	...	...		...	227
167. 2-Nitrophenylphenylchlorarsine*	...	...		...	227
168. 3-Nitrophenylphenylchlorarsine*	...	...		...	227
169. 4-Nitrophenylphenylchlorarsine*	...	...		...	227
170. <i>bis</i> (2-Chlorophenyl)chlorarsine	301e	mp	73–75°	301e	227
171. <i>bis</i> (4-Chlorophenyl)chlorarsine*	...	...		...	227
172. <i>bis</i> (3-Nitrophenyl)chlorarsine*	58	mp	112–113°	58	68, 79
173. 2-Phenylchlorarsinoaniline hydrochloride*	...	...		...	227
174. 3-Phenylchlorarsinoaniline hydrochloride*	...	...		...	227
175. 4-Phenylchlorarsinoaniline hydrochloride*	...	...		...	227
176. <i>bis</i> (<i>m</i> -Aminophenyl)chlorarsine dihydrochloride*	...	...		...	227

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
177. <i>bis</i> (<i>p</i> -Aminophenyl)chlorarsine dihydrochloride*	...	...		...	227
178. <i>bis</i> (<i>p</i> -Methoxyphenyl)chlorarsine*	...	...		...	227
179. Diphenylbromoarsine*	341	...		...	121
180. Diphenylcyanoarsine (DC)*	311	n_D^{20}	1.6254	244	311
	...	d^{35}	1.3327	244	...
	...	mp	31.2°	244	...
	...	vol ²⁰	0.0001–0.00015	311	...
181. 2-Chlorophenylphenylcyanoarsine*	301e	mp	40–42°	301e	227
182. 3-Chlorophenylphenylcyanoarsine*	...	...		...	227
183. 4-Chlorophenylphenylcyanoarsine*	301e	mp	102°	301e	227
184. 2-Nitrophenylphenylcyanoarsine*	...	...		...	227
185. 3-Nitrophenylphenylcyanoarsine*	...	...		...	227
186. 4-Nitrophenylphenylcyanoarsine*	...	...		...	227
187. <i>bis</i> (2-Chlorophenyl)cyanoarsine*	301e	mp	85–87°	301e	227
188. <i>bis</i> (4-Chlorophenyl)cyanoarsine*	...	...		...	227
189. <i>bis</i> (3-Nitrophenyl)cyanoarsine*	58	mp	151–152°	58	68, 79
190. 2-Phenylcyanoarsinoaniline*	...	...		...	227
191. 3-Phenylcyanoarsinoaniline*	...	...		...	227
192. 4-Phenylcyanoarsinoaniline*	...	...		...	227
193. <i>bis</i> (<i>m</i> -Aminophenyl)cyanoarsine*	...	...		...	227
194. Diphenylthiocyanoarsine*	58, 301j	n_D^{29}	1.6766	58	68, 79
	...	d^{27}	1.379	58	...
	...	bp ^{4.5}	217–219°	58	...
195. <i>bis</i> (<i>m</i> -Nitrophenyl)thiocyanoarsine	58	mp	103–105°	58	68, 79
196. Phenyl- <i>o</i> -tolylcyanoarsine*	301e	...		...	227
197. <i>o</i> -Chlorophenyl- <i>o</i> -tolylcyanoarsine*	...	...		...	227
198. <i>p</i> -Chlorophenyl- <i>o</i> -tolylcyanoarsine*	...	...		...	227
199. <i>o</i> -Nitrophenyl- <i>o</i> -tolylcyanoarsine*	...	...		...	227
200. <i>m</i> -Nitrophenyl- <i>o</i> -tolylcyanoarsine*	...	...		...	227
201. <i>p</i> -Nitrophenyl- <i>o</i> -tolylcyanoarsine*	...	...		...	227
202. Phenyl- <i>m</i> -tolylcyanoarsine*	...	...		...	227
203. Phenyl- <i>p</i> -tolylchlorarsine*	301e	...		...	227
204. Phenyl- <i>p</i> -tolylcyanoarsine*	301e	...		...	227
205. <i>o</i> -Chlorophenyl- <i>p</i> -tolylcyanoarsine*	...	...		...	227
206. <i>m</i> -Chlorophenyl- <i>p</i> -tolylcyanoarsine*	...	...		...	227
207. <i>p</i> -Chlorophenyl- <i>p</i> -tolylcyanoarsine*	...	...		...	227
208. <i>p</i> -Nitrophenyl- <i>p</i> -tolylcyanoarsine*	...	...		...	227
209. <i>m</i> -(Phenylcyanoarsino)benzaldehyde*	...	...		...	227
210. <i>o</i> -(Phenylchlorarsino)benzoic acid*	...	...		...	227
211. Methyl <i>o</i> -(phenylchlorarsino)benzoate*	...	...		...	227
212. <i>m</i> -(Phenylchlorarsino)benzoic acid*	301b, 301j	mp	134–136°	301b	301h
213. Methyl <i>m</i> -(phenylchlorarsino)benzoate*	301b, 301j	...		...	301h
214. Methyl <i>m</i> -(phenylcyanoarsino)benzoate*	301b, 301j	...		...	301h
215. <i>p</i> -(Phenylchlorarsino)benzoic acid*	301b, 301j	mp	115–117°	301b	301h
216. Methyl <i>p</i> -(phenylchlorarsino)benzoate*	301b, 301j	...		...	301h
217. Methyl <i>p</i> -(phenylcyanoarsino)benzoate*	301b, 301j	...		...	301h
218. 2,4-Dimethylphenylphenylcyanoarsine*	...	...		...	227
219. <i>bis</i> (<i>o</i> -Tolyl)cyanoarsine*	301e	mp	74°	301e	227
220. <i>bis</i> (<i>o</i> -Carbomethoxyphenyl)chlorarsine*	...	...		...	227
221. <i>o</i> -Tolyl- <i>m</i> -tolylcyanoarsine*	...	...		...	227
222. <i>o</i> -Tolyl- <i>p</i> -tolylcyanoarsine*	...	...		...	227
223. <i>bis</i> (<i>m</i> -Tolyl)cyanoarsine*	...	...		...	227
224. <i>m</i> -Tolyl- <i>p</i> -tolylcyanoarsine*		...		...	227
225. <i>bis</i> (<i>p</i> -Tolyl)cyanoarsine	301e	mp	62°	301e	227
226. <i>m</i> -(Phenylchlorarsino)acetophenone*	301j	mp	71–72°	301j	227
227. <i>m</i> -(Phenylcyanoarsino)acetophenone*	301j	mp	57–59°	301j	227
228. <i>m</i> -(Phenylchlorarsino)- ω -chloroacetophenone*	301j	...		...	227
229. <i>m</i> -(Phenylcyanoarsino)- ω -chloroacetophenone*	301j	...		...	227
230. 2,4-Dimethylphenyl- <i>o</i> -tolylcyanoarsine*	...	...		...	301f
231. 2,4-Dimethylphenyl- <i>p</i> -tolylcyanoarsine*	...	...		...	301f
232. α -Naphthylphenylchlorarsine*	...	...		...	227
233. β -Naphthylphenylchlorarsine*	...	...		...	227

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference to toxicity data
		Property	Reference	
234. α -Naphthylphenylecyanoarsine*	301e	mp	98–99°	301e 227
235. β -Naphthylphenylecyanoarsine*	...	...		... 227
236. α -Naphthyl- <i>p</i> -tolylecyanoarsine*	...	...		... 227
237. β -Naphthyl- <i>p</i> -tolylecyanoarsine*	...	...		... 227
238. <i>bis</i> (α -Naphthyl)chlorarsine	301j	mp	163–165°	301j ...
239. <i>bis</i> (α -Naphthyl)cyanoarsine*	301j	mp	191°	301g 301g
240. <i>bis</i> (β -Naphthyl)chlorarsine*	...	...		... 227
241. <i>bis</i> (β -Naphthyl)cyanoarsine*	...	...		... 227
242. Phenyl-(<i>x</i>)-thienylchlorarsine*	301e	bp ^{0.7}	150–156°	301e 227
243. Phenyl-(<i>x</i>)-thienylecyanoarsine*	301e	mp	49–51°	301e 227
	...	bp ^{0.5}	168–174°	301e ...
244. Phenyl-3-pyridylchlorarsine hydrochloride*	...	...		... 227
245. Phenyl-3-pyridylchlorarsine methiodide*	...	...		... 227
246. Phenyl-3-pyridylecyanoarsine hydrochloride*	...	...		... 227
247. 5-Phenylchlorarsino)-2-chloropyridine hydrochloride*	...	...		... 227
248. 5-(Phenylchlorarsino)-2-aminopyridine dihydrochloride*	...	...		... 227
249. <i>bis</i> (α -Furyl)chlorarsine*	1, 25, 301d	n_D^{28}	1.6082	25 68, 79
	...	d^{10}	1.5909	25 ...
	...	bp ¹	122–127°	25 ...
250. <i>bis</i> (α -Furyl)cyanoarsine*	39, 301e	n_D^{25}	1.5749	39 68, 79
	...	d^{25}	1.4857	39 ...
	...	bp ^{2.4}	142°	39 ...
251. <i>bis</i> (α -Thienyl)chlorarsine*	...	...		... 227
252. <i>bis</i> (α -Thienyl)cyanoarsine*	301e	mp	51–55°	301e 227
	...	bp ^{0.65}	180–182°	301e ...
253. <i>bis</i> (3-Pyridyl)chlorarsine dihydrochloride*	58	mp	270–273°	58 68
254. <i>bis</i> (3-Pyridyl)cyanoarsine*	...	...		... 227
255. <i>p</i> -Phenylenchlorarsine	1	mp	ca. 145°	1 ...
256. 1-Chlorarsindole*	...	...		... 227
257. 2-(Diethylaminomethyl)-1,3-dichlorarsindole hydrochloride	73	mp	204.2–205.2°	73 68
258. 5-Chlorodibenzarsenole*	5, 307b	mp	161°	5 68
	...	bp ²⁵	230°	5 ...
259. 5-Chloro-3,7-dinitrodibenzarsenole*	...	...		... 227
260. 5-Chloro-3-aminodibenzarsenole*	...	...		... 227
261. 5-Chloro-3,7-diaminobenzarsenole hydrochloride*	...	...		... 227
262. 5-Cyanodibenzarsenole*	5	mp	178°	5 68
263. 5-Iododibenzarsenole*	...	...		... 227
264. 4,4'-Diphenylenechlorarsine*	...	...		... 227
265. 5-Chloro-5,10-dihydroacridarsine*	39, 307b	mp	113–114°	39 227
266. 2,5-Dichloro-5,10-dihydroacridarsine*	307m	mp	116°	291e, 307m 291e
267. 5-Cyano-5,10-dihydroacridarsine*	307k	mp	114–115°	307k 285, 291d
268. 2-Chloro-5-cyano-5,10-dihydroacridarsine*	307m	mp	113–114°	307m 285, 291e
269. 5-Chloro-10-oxo-5,10-dihydroacridarsine*	...	...		... 227
270. 5-Chloro-2-methyl-5,10-dihydroacridarsine*	307j	mp	87°	307j 285
271. 5-Cyano-2-methyl-5,10-dihydroacridarsine	...	mp	87°	291f 285, 291f
272. 5-Chloro-3-methyl-5,10-dihydroacridarsine*	...	mp	65.5–66.5°	307n 291c
273. 10-Acetyl-5,10-dihydrophenarsazine*	...	...		... 227
274. 10-Acetyl-5,10-dihydrophenarsazine picrate*	...	...		... 227
275. 10-Trichloroacetyl-5,10-dihydrophenarsazine*	...	...		... 227
276. 10-Chloro-5,10-dihydrophenarsazine (DM)*	38, 73, 311	d^{20}	1.648	311 68, 79, 311, 104
	...	mp	189–190.4°	73 ...
	...	vol ²⁰	0.00002	31 ...
277. 5-Acetyl-10-chloro-5,10-dihydrophenarsazine*	...	...		... 227
278. 5-Propionyl-10-chloro-5,10-dihydrophenarsazine*	...	...		... 227
279. 5-Benzoyl-10-chloro-5,10-dihydrophenarsazine*	...	...		... 227
280. 10-Bromo-5,10-dihydrophenarsazine*	...	...		... 227
281. 10-Iodo-5,10-dihydrophenarsazine*	...	...		... 227
282. 10-Cyano-5,10-dihydrophenarsazine (Cyan DM)*	107, 108	...		... 121
283. 10-Thiocyano-5,10-dihydrophenarsazine*	...	...		... 227

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference to toxicity data
		Property	Reference	
284. 1(or 3),10-Dichloro-5,10-dihydrophenarsazine*	...	...	...	227
285. 2,10-Dichloro-5,10-dihydrophenarsazine*	...	...	...	227
286. 10-Chloro-5,10-dihydro-4 (?) -nitrophenarsazine*	...	...	...	227
287. 1(or 3),2,10-Trichloro-5,10-dihydrophenarsazine*	...	...	...	227
288. 1,3,10-Trichloro-5,10-dihydrophenarsazine*	...	...	...	227
289. 1,9(or 3,7),10-Trichloro-5,10-dihydrophenarsazine*	...	...	...	227
290. 2,8,10-Trichloro-5,10-dihydrophenarsazine*	...	...	...	227
291. 1,2,3,10-Tetrachloro-5,10-dihydrophenarsazine*	...	...	...	227
292. 2,4,6,8,10-Pentabromo-5,10-dihydrophenarsazine*	...	...	...	227
293. 2-Amino-10-chloro-5,10-dihydrophenarsazine hydrochloride*	...	...	...	227
294. 1(or 3)-Amino-10-chloro-5,10-dihydrophenarsazine hydrochloride*	...	...	...	227
295. 4-Amino-10-chloro-5,10-dihydrophenarsazine hydrochloride*	...	...	...	227
296. 10-Chloro-5,10-dihydro-1(or 3)-methylphenarsazine*	...	...	...	227
297. 10-Chloro-5,10-dihydro-2-methylphenarsazine*	...	...	...	227
298. 5-Acetyl-10-chloro-5,10-dihydro-2-methylphenarsazine*	...	...	...	227
299. 10-Chloro-5,10-dihydro-4-methylphenarsazine*	...	...	...	227
300. 1(or 3),10-Dichloro-5,10-dihydro-6-methylphenarsazine*	...	...	...	227
301. 4-Amino-10-chloro-5,10-dihydro-7-methylphenarsazine hydrochloride*	...	...	...	227
302. 10-Chloro-5,10-dihydrophenarsazine-1(or 3) carboxylic acid*	...	...	...	227
303. 10-Chloro-5, 10-dihydrophenarsazine-4-carboxylic acid*	...	...	...	227
304. 10-Chloro-5,10-dihydro-1(or 3)-6-dimethylphenarsazine*	...	...	...	227
305. 10-Chloro-5,10-dihydro-2,8-dimethylphenarsazine*	...	...	...	227
306. 5-Acetyl-10-chloro-5,10-dihydro-2,8-dimethylphenarsazine*	...	...	...	227
307. 1(or 3)-Acetyl-10-chloro-5,10-dihydrophenarsazine*	...	...	...	227
308. 1(or 3)-Aceto-10-bromo-5,10-dihydrophenarsazine*	...	...	...	227
309. 10-Chloro-5,10-dihydro-1,4,7-trimethylphenarsazine*	...	...	...	227
310. 10-Chloro-5,10-dihydro-2,4,7-trimethylphenarsazine*	...	...	...	227
311. 10-Chloro-5,10-dihydro-1(or 3)-propionylphenarsazine*	...	...	...	227
312. 12-Chloro-7,12-dihydrobenz (a) phenarsazine*	...	...	...	227
313. 7-Chloro-7,12-dihydrobenz (c) phenarsazine*	...	...	...	...
314. 12-Chloro-7,12-dihydroquino (5,6,b) benzarsazine*	...	...	...	227
315. 12-Chloro-7,12-dihydro-10-methylbenz (a) phenarsazine*	...	...	...	227
316. 7-Chloro-7,12-dihydro-9-methylbenz (c) phenarsazine*	...	...	...	227
317. 14-Chloro-7,14-dihydrodibenz-(a,j) phenarsazine*	...	...	...	227
318. 7-Chloro-7,14-dihydrodibenz-(c,h) phenarsazine*	...	...	...	227
319. 5,13-Dichloro-5,8,13,14-tetrahydro (1,4) benzarsazino (2,3,a) phenarsazine*	...	...	...	227
320. 12,14-Dichloro-5,7,12,14-tetrahydro (1,4) benzarsazino (3,2,b) phenarsazine*	...	...	...	227
321. 2,2'-bis(10-chloro-5,10-dihydrophenarsazine)*	...	...	...	227
322. 10-Chlorophenoxarsine*	288a, 307b	mp	120°	307b
323. 4,10-Dichlorophenoxarsine*	...	...	...	227
324. 10-Cyanophenoxarsine*	...	...	...	227
325. 12-Chloro-5,12-dihydrobenz (i) phenoxarsine*	...	...	...	227
326. 10-Chlorophentharsine*	...	...	...	227
327. 5,10-Dichloro-5,10-dihydroarsanthrene*	4, 110	mp	185-186°	4
328. 2,5,10-Trichloro-5,10-dihydroarsanthrene*	...	...	...	227
329. 2-Amino-5,10-dichloro-5,10-dihydroarsanthrene hydrochloride*	...	...	...	227
330. 5,10-Dicyano 5,10-dihydroarsanthrene*	...	...	...	227
331. 5,10-Dichloro-5,10-dihydro-5-arsa-10-stibanthrene*	...	...	...	227

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
<i>Tertiary arsines</i>					
332. <i>bis</i> (Chloromethyl)methylarsine*	...	...		...	227
333. 2-Chloroethyldimethylarsine*	307p	bp	100–103°	307p	227
334. <i>bis</i> (2-Chlorovinyl)methylarsine	58	n_D^{28}	1.5665	58	68, 79
	...	d^{28}	1.473	58	...
	...	bp ¹⁸	97–103°	58	...
	...	vol ²⁰	1.72	70	...
335. <i>bis</i> (2-Chloroethyl)methylarsine*	307p	bp ⁶⁵	50–55°	307p	227
336. <i>bis</i> (2-Iodoethyl)methylarsine	307p	bp ¹³	68–69°	307p	...
337. <i>bis</i> (2-Ethoxyethyl)methylarsine	58	n_D^{28}	1.4664	58	68, 79
	...	d^{25}	1.100	58	...
	...	bp ²⁰	124–125°	58	...
	...	bp ⁷²⁴	230°	58	...
338. <i>tris</i> (2-Chlorovinyl)arsine*	73	...		...	227
339. <i>tris</i> (2,2-Dichlorovinyl)arsine*	...	...		...	227
340. Triethylarsine	102	...		...	68, 79
341. 2-Chloroethyldiethylarsine*	307p	bp ²⁰	53°	307p	227
342. 2-Bromoethyldiethylarsine*	...	...		...	227
343. <i>bis</i> (2-Chloroethyl)ethylarsine*	307p	bp ¹²	64°	307p	227
344. <i>tris</i> (Cyclohexyl)arsine	39	bp ²	187–189°	39	68, 79
345. Trioctylarsine	101a	d^{19}	0.9357	101a	68, 79
	...	bp ¹⁰	238–240°	101a	...
	...	bp ¹	184–185°	101a	...
346. Phenylarsenophosgene	84i	...		...	...
347. 2-(<i>p</i> -Dimethylarsinophenyl)quinoline	84j	...		...	...
348. <i>bis</i> (2-Chlorovinyl)phenylarsine*	1, 231	d^{20}	1.384	1	68, 231
	...	bp ¹⁹	166–171°	231	...
349. 2-Chlorovinyl diphenylarsine*	1	d^{20}	1.327	1	68
350. Triphenylarsine	73	mp	58–60.5°	73	...
351. <i>tris</i> (<i>m</i> -Nitrophenyl)arsine	7	...		...	...
352. <i>tris</i> (<i>p</i> -Dimethylaminophenyl)arsine sulfur monochlo- ride addition product*	...	...		...	227
353. Tri- <i>p</i> -tolylarsine*	...	...		...	227
354. Dimethyl-2-pyridylarsine	84j	...		...	68, 79
355. Difurylmethylarsine*	301e	...		...	267
356. 2-Chlorovinyl- <i>bis</i> (2-furyl)arsine	267	bp ³	127°	267	267
357. <i>tris</i> (2-Furyl)arsine*	25, 301d	d^{29}	1.436	25	27
	...	mp	33.5°	25	...
	...	bp ³	153°	25	...
	...	bp ^{4.9}	162.5°	25	...
358. <i>tris</i> (5- <i>tert</i> -Butyl-2-furyl)arsine	101b	mp	53°	101b	68, 79
	...	bp ¹	168°	101b	...
359. <i>tris</i> (2-Thienyl)arsine*	39, 301e	n_D^{27}	1.6943	39	68, 79
	...	d^{27}	1.509	39	...
	...	bp ^{2.5}	227–230°	39	...
360. <i>tris</i> (2-Pyridyl)arsine	84c	mp	79–81°	101c	68, 79
361. <i>tris</i> (4-Dibenzofuryl)arsine	84h	...		...	...
362. <i>tris</i> (4-Dibenzothienyl)arsine	84g	mp	328–329°	101d	...
363. <i>tris</i> (<i>N</i> -Ethyl-2-carbazolyl)arsine	84d	...		...	...
364. <i>tris</i> (<i>N</i> -Ethyl-3-carbazolyl)arsine	84h	...		...	...
365. <i>tris</i> (4-Phenoxthienyl)arsine	84g	...		...	68, 79
366. 5,10-Dihydro-10-methylphenarsazine*	...	...		...	227
367. 5,10-Dihydro-10-ethylphenarsazine*	...	...		...	227
368. 5,10-Dihydro-10-phenylphenarsazine*	...	...		...	227
369. <i>bis</i> (Dimethylarsino)acetylene	73	n_D^{24}	1.5662	73	68, 79
	...	d^{26}	1.458	73	...
	...	bp ²⁹	96–98°	73	...
	...	bp ¹⁴	84.5°	73	...
370. <i>p</i> -Phenylene- <i>bis</i> (dimethylarsine)	84k	...		...	68, 79
<i>Quaternary arsenic derivatives</i>					
371. <i>o</i> -Dimethylarsinophenyl- <i>N</i> -methylcarbamate methiodide	84t	mp	130–132°	84t	...

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
372. <i>m</i> -Dimethylarsinophenyl-N-methylcarbamate methiodide	84u	mp	187–189°	84u	...
373. <i>p</i> -Dimethylarsinophenyl-N,N-dimethylcarbamate methiodide	84t	mp	226.5°	84t	...
374. <i>m</i> -Diethylarsinophenol methiodide	84v	mp	87–89°	84v	80
375. <i>m</i> -Diethylarsinophenyl-N-methylcarbamate methiodide	84v	...		...	...
376. 5,10-Dihydro-10-methylphenarsazine methiodide*	...	...		...	227
377. Tetraphenylarsonium chloride monohydrate	73	mp	262–263.5°	73	...
<i>Arsenic analogs of hydrazine</i>					
378. <i>bis</i> (Dimethylarsine) (cacodyl)	316	...		...	...
379. <i>bis</i> (Diethylarsine) (ethyl cacodyl)	17	...		...	...
380. <i>bis</i> (Phenyl-3-pyridylarsine)*	...	...		...	227
381. <i>bis</i> (2-Amino-3-pyridylphenylarsine)*	...	...		...	227
382. 10,10'- <i>bis</i> (5,10-Dihydrophenarsazine)*	...	...		...	227
383. 10,10'- <i>bis</i> (5-Acetyl-5,10-dihydrophenarsazine)*	...	...		...	227
384. 10,10'- <i>bis</i> (5,10-Dihydrophenarsazine) sulfate*	...	...		...	227
385. 2,2',4,4',6,6'-Hexanitroarsenobenzene	7	...		...	...
386. 4,4'-Dihydroxy-3,3'-dinitroarsenobenzene	7	...		...	...
387. 4,4'-Dihydroxy-3,3',5,5'-tetranitroarsenobenzene	7	...		...	...
<i>Derivatives of arsenic oxides, sulfides, and amines</i>					
388. Arsenic oxide*	...	...		...	227
389. Ethoxydichlorarsine	84l	...		...	68, 79
390. Dimethylaminodifluoroarsine	...	...		...	68
391. Isopropoxydichlorarsine	84m	bp ⁷³⁷	155–156°	84m	...
392. 2-Chloro-4,5-dihydro-1,3,2-oxthiarsenole	84m	<i>n</i> _D ²⁴	1.6690	84m	68, 79
	...	<i>d</i> ²⁵	1.988	84m	...
	...	bp ^{0.3}	72–73°	84m	...
393. Diethoxychlorarsine	84l	...		...	68, 79
394. <i>bis</i> (2-Chloroethoxy)chlorarsine	47	bp ^{1.2}	112–118°	47	68
395. Diisopropoxyfluoroarsine	84j	...		...	...
396. <i>tris</i> (2-Fluoroethoxy)arsine	86	...		...	68
397. <i>tris</i> (2-Chloroethoxy)arsine	47	bp ¹⁰	160–170°	47	68
398. <i>tris</i> (2-Chloroethylthio)arsine*	84r	<i>d</i> ²⁰	1.5972	84r	68
399. <i>tris</i> (Phenylthio)arsine	84o	mp	92–94°	84o	68
400. Methylarsine disulfide*	...	...		...	227
401. Phenylmethoxychlorarsine*	...	...		...	227
402. Phenylethoxychlorarsine*	...	...		...	227
403. Phenyl-2-chloroethylthiochlorarsine*	297a	...		...	227
404. <i>o</i> -Hydroxychlorarsinobenzoic anhydride*	58	mp	146.5–147°	58	68
	...	bp ³⁷	233–236°	58	...
405. <i>o</i> -Phenylenediarsine oxychloride*	73	mp	150.5–151.5°	73	68
406. Methylarsenic oxide*	311	mp	95°	311	311
	...	bp	ca. 275°	311	...
407. Methylarsenic sulfide*	...	...		...	227
408. Methyldimethoxyarsine*	...	...		...	227
409. Methyldimethylthioarsine*	...	...		...	227
410. Methyldiethoxyarsine*	...	...		...	227
411. Methyl- <i>bis</i> (N,N-diethyldithiocarbamyl)arsine*	...	...		...	227
412. Methyl- <i>bis</i> (N,N- <i>bis</i> (2-hydroxyethyl)dithiocarbamyl)arsine*	...	...		...	227
	...	...		...	227
413. Methyldiphenylthioarsine*	...	...		...	227
414. Methyldi- <i>p</i> -tolylthioarsine*	...	...		...	227
415. 2-Chlorovinylarsenic oxide (various isomers)*	12, 311	Physical properties vary with method of preparation		122, 12	68, 79
				...	227
416. 2-Chlorovinylarsenic sulfide*	...	...		...	227
417. 2-Chlorovinylarsenic selenide	67	...		...	68
418. 2-Chlorovinylldimethoxyarsine	...	...		...	68, 79

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference to toxicity data
		Property	Reference	
419. 2-Chlorovinyl dimethylthioarsine	...	...	...	68, 79
420. 2-Chlorovinyl diethoxyarsine*	30	bp ¹⁷⁻¹⁸	30	68, 79
421. 2-Chlorovinyl- <i>bis</i> (2-chloroethylthio)arsine*	84p	n_D^{20}	84p	68, 79
	...	d^{20}	84p	...
	...	bp ¹	84p	...
422. 2-Chlorovinyl- <i>bis</i> (2-ethoxyethoxy)arsine*	...	...	...	227
423. 2-Chlorovinyl- <i>bis</i> (2-hydroxyethylthio)arsine	33	bp ^{2.5-3}	33	68
424. 2-Chlorovinyl diallyloxyarsine*	...	...	...	227
425. 2-Chlorovinyl diisopropoxyarsine*	...	...	...	227
426. 2-Chlorovinyl dipentoxarsine*	...	...	...	227
427. 2-(2-Chlorovinyl)-5,5- <i>bis</i> (hydroxymethyl)-4,5-dihydro-1,3,2-dithiarsin	33	mp	33	68
428. 2-Chlorovinyl diisooctyloxyarsine*	...	...	...	227
429. 2-Chlorovinyl diphenylthioarsine*	...	...	...	227
430. 2-Chlorovinyl- <i>bis</i> (N,N-diethyldithiocarbamyl)arsine*	...	...	...	227
431. Ethylarsenic oxide*	103a, 311	$n_D^{11.5}$	311	68, 79
	...	$d^{11.5}$	311	...
	...	bp ¹	103a	...
432. Ethyldimethylthioarsine	...	...	...	68
433. Ethyl- <i>bis</i> (2-chloroethoxy)arsine	103b	...	...	68
434. Ethyldipropoxyarsine	58	$n_D^{30.5}$	58	68, 79
	...	d^{27}	58	...
	...	bp ¹⁸	58	...
	...	bp ⁷³⁸	58	...
435. N-Ethylethylarsenimide	58	n_D^{26}	58	68, 79
	...	d^{25}	58	...
	...	bp ^{3.5}	58	...
436. Propyldiacetoxyarsine	58	n_D^{26}	58	68, 79
	...	d^{26}	58	...
	...	bp ¹³	58	...
437. Amylarsenic oxide	35	...	...	...
438. Isoamylarsenic oxide	35	...	...	...
439. Hexylarsenic oxide	35	...	...	...
440. Phenylarsenic oxide*	39, 311	mp	39	68, 291a
		(But varies with method of preparation)	311	
441. <i>o</i> -Nitrophenylarsenic oxide	7	...	...	...
442. <i>m</i> -Nitrophenylarsenic oxide	58	mp	58	68, 79
443. 2,4,6-Trinitrophenylarsenic oxide	7	...	...	...
444. 2,4,6-Trinitrophenylarsenic dinitrate	7	...	...	...
445. Phenyl- <i>bis</i> (2-chloroethylthio)arsine*	297a	...	...	227
446. <i>p</i> -Hydroxyphenylarsenic oxide*	...	...	...	227
447. <i>m</i> -Aminophenylarsenic oxide*	...	...	...	227
448. <i>p</i> -Dimethylaminophenylarsenic oxide*	7	...	...	291b
449. 3-Amino-4-hydroxyphenylarsenic oxide hydrochloride (Mapharsen)	Commercial	...	...	334
450. <i>o</i> -Arsenosobenzoic acid*	...	...	...	227
451. <i>m</i> -Arsenosobenzoic acid*	...	...	...	227
452. 3-Pyridylarsenic oxide*	...	...	...	227
453. 2-Chloropyridine-5-arsenic oxide*	...	...	...	227
454. 2-Dibenzothienylarsenic oxide	84f	...	...	...
455. 4,4'- <i>bis</i> (Arsenoso)biphenyl*	...	...	...	227
456. <i>bis</i> (Dimethylarsenic) oxide (cacodyl oxide)	8, 38	bp	38	68, 79
457. 2-Chloroethylthiodimethylarsine	84m	bp ¹⁵	84m	68, 79
458. <i>bis</i> (Dimethylarsenic) disulfide*	...	...	...	227
459. <i>bis</i> (Diethylarsenic) oxide	17	...	...	...
460. <i>bis</i> (<i>bis</i> -2-Chlorovinylarsenic) oxide	...	...	...	235
461. <i>bis</i> (2-Chlorovinyl)-2-chloroethylthioarsine	84q	n_D^{26}	84q	68
	...	bp ^{0.5}	84q	...
462. <i>bis</i> (2-Chlorovinyl)-1,3-dichloropropyl-2-thioarsine	84s	bp ^{0.2}	84s	68

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties			Reference to toxicity data
		Property	Reference		
463. Methylphenylmethoxyarsine	58	n_D^{30}	1.5613	58	68, 79
	...	d^{31}	1.295	58	...
	...	bp ¹⁶⁻¹⁷	101-102°	58	...
464. Methylphenylacetoxyarsine	58	n_D^{29}	1.5612	58	68, 79
	...	d^{28}	1.369	58	...
	...	bp ¹⁴	140-142°	58	...
465. 2-Chloroethylthiodiphenylarsine*	...	...		...	227
466. Benzoxydiphenylarsine*	...	...		...	227
467. <i>bis</i> (Diphenylarsenic) oxide*	...	...		...	227
468. <i>bis</i> (Diphenylarsenic) sulfide*	301j	mp	58-60°	301j	227
469. <i>bis</i> (<i>bis</i> -(<i>p</i> -Aminophenyl) arsenic) oxide*	...	...		...	227
470. <i>o</i> -Phenylhydroxyarsinobenzoic acid anhydride*	...	...		...	227
471. <i>bis</i> (<i>o</i> -Carboxyphenyl) hydroxyarsine anhydride*	...	...		...	227
472. <i>bis</i> (<i>o</i> -Carboxyphenylphenylarsenic) oxide*	...	...		...	227
473. <i>bis</i> (<i>p</i> -Carboxyphenylphenylarsenic) oxide*	...	...		...	227
474. <i>p</i> -Phenylene- <i>bis</i> (phenylarsine) monoxide*	...	...		...	227
475. Acetoxydithienylarsine*	...	...		...	227
476. 1,3-Dihydroxyarsindole*	...	...		...	227
477. <i>bis</i> (Dibenzarsenyl-5) oxide*	...	...		...	227
478. <i>bis</i> (3,7-Dinitrodibenzarsenyl-5) oxide*	...	...		...	227
479. <i>bis</i> (3-Aminodibenzarsenyl-5) oxide*	...	...		...	227
480. <i>bis</i> (Phenoxarsinyl-10) oxide*	288a	mp	182°	...	227
481. 10-Chloroethylthio-5,10-dihydrophenarsazine	84n	mp	128-153°	84n	...
482. <i>bis</i> (5,10-Dihydrophenarsazinyl-10) oxide*	...	...		...	227
483. <i>bis</i> (5,10-Dihydrophenarsazinyl-10) sulfide*	...	...		...	227
484. <i>bis</i> (5,10-Dihydrophenarsazinyl-10) oxide. 10-Chloro-5,10-dihydrophenarsazine (basic DM)*	...	...		...	227
485. <i>bis</i> (5-Acetyl-5,10-dihydrophenarsazinyl-10) oxide*	...	...		...	227
486. <i>bis</i> (5-Benzoyl-5,10-dihydrophenarsazinyl-10) oxide*	...	...		...	227
487. 5,10-Dihydroarsanthrene-5,10 monoxide*	...	...	...	...	227
488. 5,10-Dihydro-5,10-dioxystibarsanthrene-5,10 monoxide*	...	...		...	227
<i>Halogen and oxygen derivatives of tertiary arsines</i>					
489. Triphenyldichlorarsine*	...	...		...	227
490. Tri- <i>m</i> -nitrophenyldibromoarsine	7	...		...	...
491. Tri- <i>p</i> -tolyl-dichlorarsine*	...	...		...	227
492. Tri- <i>m</i> -nitrophenylarsenic oxide	7	...		...	...
493. Tri- <i>m</i> -nitrophenylarsenic dinitrate	7	mp	147-148°	7	...
494. <i>o</i> -Carboxyphenylphenylmethylarsenic oxide*	...	...		...	227
495. 7-Phenylmethyloxidoarsino-2-naphthalenesulfonic acid*	...	...		...	227
<i>Derivatives of arsenic, arsonic, and arsinic acids</i>					
496. <i>o</i> -Nitroanilinium arsenate	7	mp	146-147°	7	...
497. <i>m</i> -Nitroanilinium arsenate	7	mp	114-115°	7	...
	...	...	then 147-148°	7	...
498. <i>p</i> -Nitroanilinium arsenate	7	mp	77-78°	7	...
499. Sodium methanearsenate hydrate	69	...		...	68
500. 2-Chloroethylenearsonic acid*	69	mp	129°	69	68, 79
501. Ethanearsonic acid	69	mp	96°	69	68
502. Benzenearsonic acid	58	mp	165°	58	68, 79
503. <i>o</i> -Nitrobenzenearsonic acid	7	...		...	...
504. Cadmium <i>o</i> -nitrobenzenearsonate	34	...		...	...
505. Lead <i>o</i> -nitrobenzenearsonate	7	...		...	...
506. <i>m</i> -Nitrobenzenearsonic acid	7	...		...	...
507. Lead <i>m</i> -nitrobenzenearsonate	7	...		...	...
508. <i>p</i> -Nitrobenzenearsonic acid	7	...		...	...
509. Sodium 2,4-dinitrobenzenearsonate	7	...		...	...
510. Magnesium 2,4-dinitrobenzenearsonate	84a	...		...	...
511. Potassium 2,4-dinitrobenzenearsonate	84a	...		...	...

TABLE 9 (Continued).

Compound	Reference to synthesis	Physical properties		Reference to toxicity data
		Property	Reference	
512. Manganous 2,4-dinitrobenzenearsonate	84a	...	...	...
513. Ferric 2,4-dinitrobenzenearsonate	84a	...	...	...
514. Cobalt 2,4-dinitrobenzenearsonate	84a	...	...	...
515. Nickel 2,4-dinitrobenzenearsonate	84a	...	...	...
516. Cupric 2,4-dinitrobenzenearsonate	84a	...	...	...
517. Cadmium 2,4-dinitrobenzenearsonate	34	...	...	...
518. Stannic 2,4-dinitrobenzenearsonate	84a	...	...	...
519. Barium 2,4-dinitrobenzenearsonate	84a	...	...	...
520. Mercuric 2,4-dinitrobenzenearsonate	84a	...	...	...
521. Lead 2,4-dinitrobenzenearsonate	101b	...	...	...
522. 2,4,6-Trinitrobenzenearsonic acid	7	...	...	...
523. Sodium 2,4,6-trinitrobenzenearsonate	84a	...	...	...
524. Potassium 2,4,6-trinitrobenzenearsonate	84a	...	...	...
525. Calcium 2,4,6-trinitrobenzenearsonate	84a	...	...	...
526. Cupric 2,4,6-trinitrobenzenearsonate	84a	...	...	...
527. Cadmium 2,4,6-trinitrobenzenearsonate	34	...	...	...
528. Stannic 2,4,6-trinitrobenzenearsonate	84a	...	...	...
529. Barium 2,4,6-trinitrobenzenearsonate	84a	...	...	...
530. Mercuric 2,4,6-trinitrobenzenearsonate	84a	...	...	...
531. Lead 2,4,6-trinitrobenzenearsonate	7	...	...	...
532. <i>p</i> -Hydroxybenzenearsonic acid*	...	...	...	227
533. 4-Hydroxy-3-nitrobenzenearsonic acid	7	...	...	...
534. Lead 4-hydroxy-3-nitrobenzenearsonate	7	...	...	...
535. 4-Hydroxy-3,5-dinitrobenzenearsonic acid	7	...	...	...
536. Cadmium 4-hydroxy-3,5-dinitrobenzenearsonate	34	...	...	...
537. Lead 4-hydroxy-3,5-dinitrobenzenearsonate	7	...	...	...
538. Cadmium 2,4-dihydroxy-3,5-dinitrobenzenearsonate	34	...	...	...
539. <i>o</i> -Arsanilic acid*	...	...	...	227
540. 2-Amino-5-nitrobenzenearsonic acid*	...	...	...	227
541. <i>p</i> -Arsanilic acid picrate	7	mp	169-170°	7
542. 4-Amino-3,5-dinitrobenzenearsonic acid	7	...	...	...
543. Lead 4-amino-3,5-dinitrobenzenearsonate	7	...	...	...
544. <i>o</i> -Toluenearsonic acid*	...	...	...	227
545. 2-Biphenylarsonic acid*	...	...	...	227
546. 4,4'-Biphenyldiarsonic acid*	...	...	...	227
547. 2-Benzophenonearsonic acid*	...	...	...	227
548. 8-Methylquinoline-5-arsonic acid	84f	...	...	...
549. 2-Dibenzothiophenearsonic acid	84f	...	...	...
550. N-Ethylcarbazole-3-arsonic acid	84e	...	...	...
551. 1,3-Dihydroxy-1-oxyarsindole*	...	...	...	227
552. 5-Hydroxy-5-oxydibenzarsenole*	...	mp	7250°	5
553. 5,10-Dihydro-10-hydroxy-10-oxyphenarsazine*	...	...	...	227
554. 5,10-Dihydro-10-hydroxy-10-oxyphenarsazine hydrochloride*	...	...	...	227
555. 10,10-Dihydroxy-10-ethylphenoxarsine*	...	...	...	227
556. 5,10-Dihydro-10,10-dihydroxy-10-ethylphenarsazine*	...	...	...	227
<i>Arsenic derivatives of uncertain constitution</i>				
557. Chlorination product of Propane-1,3-diarsonic acid*	...	...	...	227
558. By-product of the preparation of dimethylaminodifluoroarsine	...	...	...	68
559. Anhydride from <i>o</i> -hydroxyphenylarsenic oxide*	...	...	...	227
560. Di(<i>bis</i> (5,10-dihydrophenarsazine-10)oxalate)-acetate*	...	...	...	227

No compounds of unusual toxicity were discovered in this class, and, although some of the members appeared to offer potentialities as irritant smokes, none showed evidence of being significantly better than the standard irritants DM, DA, and DC.

7.3.4 Irritant Arsenical Smokes

Certain arsenical compounds which are relatively nonvolatile and of fairly low toxicity are, nevertheless, highly irritating to the respiratory tract when dispersed as a cloud of very fine particles. Further-

more, such particles will penetrate gas mask canisters unless the canisters are fitted with an efficient particulate filter. Such filters were not available in World War I, but have since been developed and are standard equipment of all nations.

Diphenylchlorarsine (DA) was introduced by the Germans in 1917 as a mask-breaking irritant which was expected to produce temporary casualties and to cause troops to unmask and expose themselves to the effects of more lethal agents, usually employed simultaneously. In 1918 diphenylcyanoarsine (DC) was used for the same purpose.

The Allies claimed that these agents were not very effective but were inclined to attribute their lack of success in part to the German method of dispersal of the agents.³²⁹ The Americans produced a new respiratory irritant diphenylaminechlorarsine (DM), usually called adamsite after its discoverer, Dr. Roger Adams. DM was not used in World War I, but was adopted by the United States as their standard irritant smoke and has been found to be useful in riot control, since only temporary casualties are produced.

During World War I, the method of dispersing the irritant smokes was in artillery shell employing a large burster charge. The particles obtained by this method are too large to obtain maximal effect from the agent. The method now used consists of volatilization of the arsenical in a cloud of hot gas produced in a thermal generator. The hot arsenical vapor condenses to a cloud of very minute particles on contact with the air.

The Japanese used irritant smoke candles on a number of occasions against both Chinese and American troops, although the attacks were always on a small scale and were apparently undertaken by group commanders without the sanction of the high command.

PHYSIOLOGICAL ACTION

The physiological action of the irritant smokes has been summarized in the open literature.³²⁹ The effects of exposure consist of severe irritation to the nose and throat resembling that from a heavy cold. There is much sneezing, watering of the eyes, and flow of mucous from the nose. Headache, pain in the ears and gums, and nausea are frequently encountered. A feeling of depression often accompanies exposure to the arsenical smokes and is thought to be largely psychological in origin.

TOXICITY

The $L(Ct)_{50}$'s by inhalation of DA, DC, and DM

have not been determined with very great accuracy for many species, but figures quoted²¹⁶ lead to the conclusion that the $L(Ct)_{50}$ for man would be greater than 10 mg min/l. Since the particulate clouds are nonpersistent, death from inhalation of the arsenical smokes could be expected only under very unusual circumstances.

British experiments²⁴⁷ have indicated that men exposed to relatively high concentrations of DC (0.00265 mg/l) for periods of 15 to 30 seconds and re-exposed to the same concentration four times at half-hourly intervals, do not show any cumulative toxic effect but rather develop some tolerance to the agent.

A number of compounds were examined by the British^{273-275,291d-291g} without revealing any more effective than DC, DA, and DM. From the effect of DC on rabbit eyes it has been concluded that a drop of <0.4 mm in diameter would probably not cause permanent damage to the human eye, but that greater contamination than this might exert a gross caustic effect which if untreated might lead to total loss of the eye.²⁷⁰

ASSESSMENT OF MILITARY VALUE

The particulate filter of modern gas masks affords adequate protection against the irritant smokes. Such agents would only be of value, therefore, if they could be used for surprise effect before the men could mask.

Acting on the suspicion that the effects of exposure to the irritant smokes was largely psychological, the British²⁵¹ carried out important experiments in 1942 in which a group of troops exposed to DA and a control group exposed to a harmless smoke were put through an assault course test. The troops were strongly motivated to turn in a good performance and were unaware of the fact that there was any difference in the two types of smoke. The group exposed to DA remained in a cloud for 2 minutes or until the generator had burned out, and the mean concentration of DA was 0.0266 mg/l. The results showed that the performance of about two-thirds of the group exposed to DA was hardly affected at all, that the performances of the remaining one-third over the assault course was definitely slower than the control group, and that about 7 per cent of the men exposed to DA were unable to complete the assault course. In view of the proximity of the men to the DA generator and the length of exposure, it was concluded that the dosage of DA was greater than could be ex-

pected in the field. Further experiment led to the following conclusions:

1. The effect on fresh troops of DA in concentrations practicable under active service conditions is almost negligible, apart from the fact that one man in ten would have great difficulty in keeping on his gas mask when doing heavy work.

2. Experiments on tired troops suggest that concentrations of DA practicable under active service conditions give an average effect which is equivalent to making fresh troops wear their gas masks.

3. Hence, DA is not an effective weapon even when used against tired men.

In view of these findings and the failure to dis-

cover an arsenical smoke significantly more effective than DA, DC, and DM, little interest remains in the irritant smokes as chemical warfare agents.

7.4 **TABULATION OF ARSENICALS EXAMINED AS CANDIDATE CHEMICAL WARFARE AGENTS**

Table 9 comprises as complete as possible a tabulation of arsenical compounds that have been examined as candidate chemical warfare agents. References to synthesis, physical properties, and toxicological screening data are included.

Chapter 8

ALIPHATIC NITROSOCARBAMATES AND RELATED COMPOUNDS^a

By Marshall Gates and Birdsey Renshaw

8.1 INTRODUCTION

A SURVEY CONDUCTED by the National Defense Research Committee [NDRC] revealed that ethyl N-methyl-N-nitrosocarbamate ("nitrosomethylurethane") was one of the most disagreeable and toxic commercially available compounds which had not received careful study in connection with chemical warfare.² Although it proved to be insufficiently toxic to compete with standard agents, synthesis and assay of related compounds revealed a number of highly toxic substances. The most promising of these was methyl N-(β -chloroethyl)-N-nitrosocarbamate (KB-16).

KB-16 is a persistent agent with a volatility only slightly less than that of mustard gas (H). Its synthesis, although more involved than that of H, of lewisite (L), or of the nitrogen mustards, presents no great difficulty and the required starting materials are readily available.

KB-16 came under investigation in 1942 at a time when the nitrogen mustards were being seriously considered. It was quickly shown that the compound possesses some of the desirable characteristics of methyl-*bis*(β -chloroethyl)amine (HN2) — low freezing point, lack of pronounced odor, and effectiveness as an eye-injurer at low dosages. Interest was also aroused by the finding that for mice it is three times as toxic as H.

Subsequent investigations revealed that: (1) KB-16 possesses inadequate storage stability, and no satisfactory stabilizer has been found in spite of intensive search; (2) its eye-injuring potency is not of a different order from that of H or *tris*(β -chloroethyl)amine (HN3); (3) although more toxic than H and the nitrogen mustards for mice, it is not so toxic as these substances for larger species (i.e., dogs, goats, and monkeys); and (4) as a vesicant it is markedly inferior to H. Taking these and other findings into account, assessment of the merits and limitations of KB-16 led to the conclusion that it does not possess the general utility of the standard agent, H, or of the potentially available nitrogen mustard, HN3. Ac-

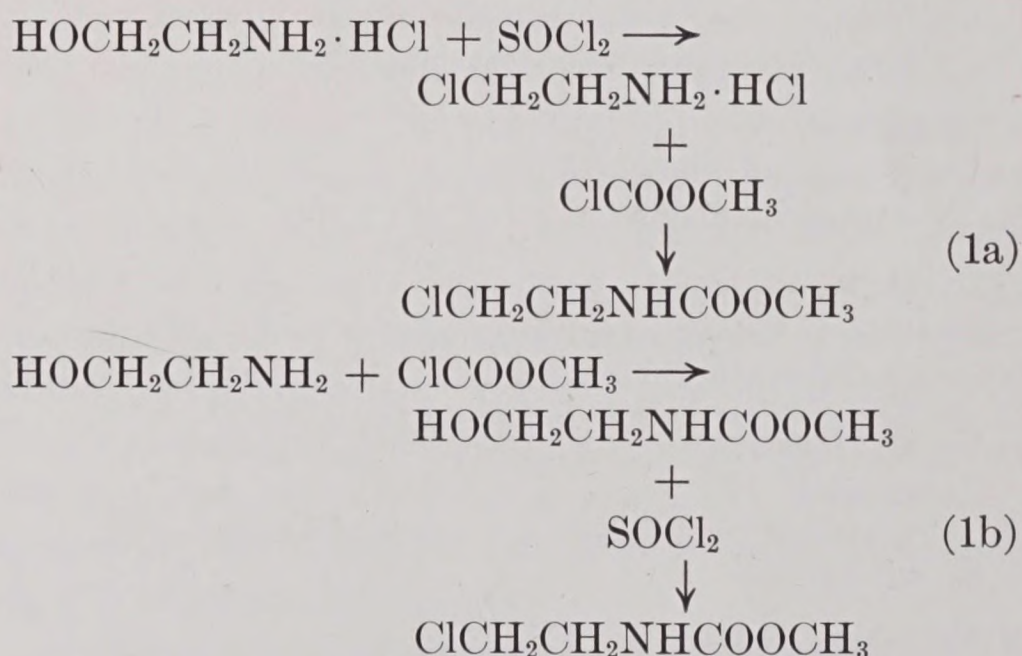
cordingly, KB-16 is not now seriously considered for use in chemical warfare.

8.2 SYNTHESIS AND PROPERTIES

8.2.1 Synthesis

The aliphatic nitrosocarbamates tested during World War II (see Table 1) were prepared by nitrosation of the corresponding carbamates, which in turn were derived from the action of alkyl chloroformates on amines. The synthesis of methyl N-(β -chloroethyl)-N-nitrosocarbamate (KB-16), the only member of the series that has received detailed study, involves the following steps.

1. Preparation of N-(β -chloroethyl)carbamate. Thionyl chloride is allowed to react with ethanolamine hydrochloride to produce β -chloroethylamine hydrochloride, which is then treated with methyl chloroformate. Alternatively, methyl chloroformate can be treated with ethanolamine and the resulting methyl N-(β -hydroxyethyl)carbamate converted to the desired product by the use of thionyl chloride. The first of these alternatives is preferable (see below). Attempts to prepare methyl N-(β -chloroethyl)carbamate directly by the action of methyl chloroformate on ethyleneimine have not succeeded.⁴³



Methyl chloroformate may be prepared in good yield either by the addition of methanol to an excess of liquid phosgene^{2,43,49} or by the reverse addition of excess gaseous phosgene to methanol.^{23b} The second alternative gives better yields based on methanol. An excess of phosgene is required to mini-

^a Based on information available to NDRC Division 9 as of November 1, 1945.

TABLE 1. Aliphatic nitrosocarbamates and related compounds examined as candidate chemical warfare agents.

The compounds are arranged in four major categories in the following sequence: (1) nitrosocarbamates, (2) nitrosoamides, (3) nitrosoamines, and (4) miscellaneous carbamates.

The following abbreviations are used: n_D^t , refractive index at t C; d^t , density in g/ml at t C; $d_{t_2}^{t_1}$ specific gravity at t_1 C in reference to water at t_2 C; mp, melting point in C; bp^p, boiling point in C at p mm Hg; vp^t, vapor pressure in mm Hg at t C; and vol^t, saturation concentration (volatility) in mg/l at t C.

Centigrade scale is used throughout.

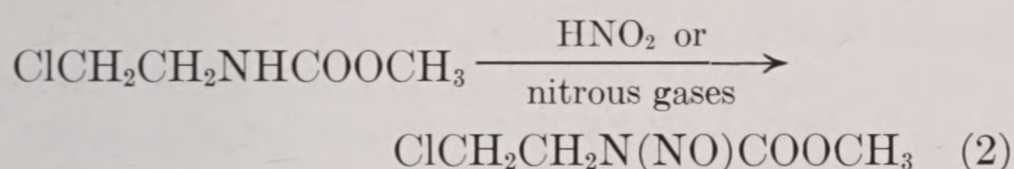
Compound	Reference to synthesis	Physical properties			Reference to toxicity data
		Property		Reference	
<i>Nitrosocarbamates</i>					
1. Methyl N-methyl-N-nitrosocarbamate	57	n_D^{20}	1.44236	48	44
	...	d^{20}	1.2105	48	...
	...	bp ¹⁵	59–60°	48	...
2. Ethyl N-methyl-N-nitrosocarbamate	Commercial	n_D^{20}	1.43632	60	10
	...	d_4^{19}	1.1402	56	...
	...	bp ¹³	65–65.5°	60	...
3. β -Fluoroethyl N-methyl-N-nitrosocarbamate	21b	bp ¹	70–80°	21e	10
4. β -Chloroethyl N-methyl-N-nitrosocarbamate	2	bp ^{0.2}	76–78°	2	10
5. Ethyl N-ethyl-N-nitrosocarbamate	Commercial	bp ²²	80–84°	...	10
6. Methyl N-(β -chloroethyl)-N-nitrosocarbamate	2, 21a, 23c, 43	n_D^{19}	1.4666	41	10, 41, 44
	...	d_4^{25}	1.3053	41	...
	...	bp ^{0.5}	72–76°	2	...
	...	vol ²⁰	0.600	11	...
7. Ethyl N-(β -chloroethyl)-N-nitrosocarbamate	2, 21a, 43	bp ¹⁰	92–93°	2	10
	...	vol ²⁰	0.426	11	...
8. β -Fluoroethyl N-(β -chloroethyl)-N-nitrosocar- bamate	21b	bp ²	118–121°	21e	10
9. Isopropyl N-(β -chloroethyl)-N-nitrosocarbamate	2	bp ^{0.5}	80°	2	10
10. Butyl N-(β -chloroethyl)-N-nitrosocarbamate	2	bp ^{0.6}	95°	2	10
11. Methyl N-(β -bromoethyl)-N-nitrosocarbamate	21d, 54c	bp ¹	110–115°	21d	10, 44
12. Methyl N-(β -hydroxyethyl)-N-nitrosocarbamate	2	bp ^{0.9}	90–95°	2	10
13. Methyl N-(β -chloropropyl)-N-nitrosocarbamate	2	bp ^{0.3–0.5}	75–80°	2	10
14. Methyl N-butyl-N-nitrosocarbamate	2	bp ²	70–72°	2	10
15. Methyl N- β -(β' -chloroethylthio)-ethyl-N-nitroso- carbamate	...	...	...	...	44
16. Methyl N-phenethyl-N-nitrosocarbamate	21d	Cannot be distilled		21d	10
<i>Nitrosoamides</i>					
17. N-(β -Chloroethyl)-N-nitrosoformamide	2	bp ^{0.8}	78–80°	2	...
18. N-(β -Chloroethyl)-N-nitrosoacetamide	2	bp ^{0.5}	70–72°	2	10
19. N-Methyl-N-nitrosofluoroacetamide	51	bp ¹⁴	84°	51	50
<i>Nitrosoamines</i>					
20. N-Nitrosopiperidine	2	bp ¹⁴	94–96°	2	10
21. N-Nitrosomorpholine	2	mp	28°	2	10
	...	bp ¹⁴	105–107°	2	...
22. N,N'-Dinitrosopiperazine	2	mp	155–157°	2	...
23. β -Chloroethylmethylnitrosoamine	54b	...	...	...	44
24. bis(β -Chloroethyl)nitrosoamine	2	Cannot be distilled		2	10, 44
25. 4-Methyl-4(methylnitrosoamino)-pentanone-2	...	...	...	...	10
26. N,N'-Dimethyl-N,N'-dinitroso- <i>p</i> -phenylene- diamine	15	mp	149–150°	15	10
27. N,N'-bis(β -Chloroethyl)-N,N'-dinitroso- <i>p</i> -phenyl- enediamine	15	mp	106.5°	15	10
<i>Miscellaneous carbamates</i>					
28. Methyl N-(β -chloroethyl)-N-nitrocarbamate	2	bp ^{0.3}	95–100°	2	10
	...	vol ²⁰	0.138	11	...
29. Methyl N- β -chloroethylcarbamate	2	n_D^{20}	1.4575	27	10, 44
	...	bp ¹⁴	100°	2	...
30. Ethyl N-isobutylcarbamate	15	bp ¹⁶	94°	15	10
31. Ethyl N-isoamylcarbamate	15	n_D^{20}	1.4333	15	10
	...	bp ¹⁶	109°	15	...
32. Ethyl N-methoxycarbamate	...	...	...	...	10

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
33. Methyl N-ethylthiocarbamate	12	$n_D^{29.5}$	1.4978	12	10
	...	d^{25}	1.078	12	...
	...	bp ²⁹	118°	12	...
	...	bp ³⁹	123°	12	...
34. Methyl N-ethylthionocarbamate	12	n_D^{25}	1.5150	12	10
	...	d^{25}	1.067	12	...
	...	bp ²⁵	109.5–110.5°	12	...
	...	bp ³⁷	119–121.5°	12	...
35. Methyl N-ethyldithiocarbamate	12	n_D^{27}	1.6139	12	10
	...	d^{25}	1.151	12	...
	...	bp ^{1.8}	121–122°	12	...

mize formation of methyl carbonate. Distillation of the crude methyl chloroformate is not necessary.⁴⁹

2. Preparation of KB-16 by nitrosation of methyl N-(β -chloroethyl)carbamate. This step may be effected by nitrous acid in solution or by nitrous gases either with or without a solvent. The action of nitrous gases on methyl N-(β -chloroethyl)carbamate in the absence of a solvent is the most rapid and convenient.



Reaction (1a) was employed in the original laboratory preparation of KB-16.^{2,21a} β -Chloroethylamine hydrochloride prepared essentially according to Ward⁶¹ from solid ethanolamine hydrochloride and thionyl chloride in chloroform was treated as a solid suspended in ether or benzene with aqueous alkali and methyl chloroformate. The resulting methyl N-(β -chloroethyl)carbamate was purified by distillation, diluted with ether or benzene, mixed with a solution of sodium nitrate, and nitrosated by the addition of nitric or sulfuric acid. Overall yields of 62 per cent were obtained. Alternatively, N-(β -chloroethyl)carbamate was nitrosated under anhydrous conditions by the use of nitrous gases. Flash distillation was used to purify the final product and appears to be the only feasible method. The above procedures were used with little modification for the synthesis of the first samples investigated in Great Britain.^{54a} The method is well suited for large-scale runs.

Preparation of KB-16 by the alternative procedure utilizing reaction (1b) is also convenient for laboratory scale work and can be carried out in overall yields of 65 per cent.^{41,43} It is less readily modified for use on a larger scale because the hydroxycar-

bamate must be distilled and the conversion of this intermediate to the chloro compound has not been achieved in yields greater than 75 per cent.

The first method, as modified for production on a larger scale, has been simplified by: (1) elimination of the isolation of ethanolamine hydrochloride and of β -chloroethylamine hydrochloride, both of which are hygroscopic; (2) combination of the first three steps into one; (3) reduction of the large excess of thionyl chloride and sodium nitrite; (4) the use of a single solvent (chloroform or benzene) in reduced quantity throughout the reaction steps; and (5) elimination of all distillations except that of the methyl N-(β -chloroethyl)carbamate.^{9,23a,49} A brief description of the modified process follows. Ethanolamine in chloroform,⁴⁹ in benzene,^{23a} or in the absence of a solvent⁹ is treated with dry hydrogen chloride to produce ethanolamine hydrochloride. Thionyl chloride is then added directly (if no solvent was used in the first step, benzene is added at this point), and the mixture is heated to convert the ethanolamine hydrochloride to β -chloroethylamine hydrochloride. The mixture is then diluted with water, and caustic alkali and methyl chloroformate are added. After the acylation is complete, the organic layer is separated, washed, dried, and stripped of solvent. The crude methyl N-(β -chloroethyl)carbamate thus obtained is then distilled under diminished pressure. It has been obtained in yields of 77.5 per cent in runs utilizing 24.5 lb of ethanolamine.^{23a}

If nitrosation is carried out by slowly acidifying a mixture of the carbamate in benzene or ether with an aqueous nitrite solution, the reaction is slow and a large excess of sodium nitrite is necessary.^{2,23a} When the reverse addition is used and the reaction mixture is strongly acid, a slight excess of sodium nitrite is sufficient and the reaction proceeds rapidly.⁴⁹

Aqueous nitrosations were used in all investigations where scaling up the synthesis of KB-16 was tried, but it was subsequently shown that the reaction of nitrous gases with methyl β -chloroethylcarbamate in the absence of a solvent is quantitative and almost instantaneous.^{21c,23c} This variation possesses a number of practical advantages.

1. Solvent is eliminated and effective reactor capacity is thereby increased.

2. The purity of the final product is sufficient to obviate the need for flash distillation.

3. Equipment is simplified and the total time cycle is reduced.

4. The method should allow preparation of the agent *in situ* shortly before use, or in shell subsequent to firing. This would eliminate the problem of storage stability and greatly lessen the hazards involved in synthesis.

Preliminary design data and cost estimates for a plant to produce KB-16 at the rate of 500 tons per month have been submitted. The calculations were based on the use of aqueous nitrosation in the final step.⁹

N-(β -Chloroethyl)-N-nitrosoacetamide, a highly toxic analog of KB-16, has been prepared on a laboratory scale by nitrosation of an ethereal solution of N-(β -chloroethyl)acetamide with oxides of nitrogen. Yields of 60 per cent of material purified by flash distillation were obtained.²

8.2.2 Physical Properties

KB-16 is usually obtained as an orange-red limpid oil of limited thermal stability. It is soluble in water to the extent of 0.7 g/100 g, and is completely miscible with ordinary organic solvents. Although it has not been obtained in crystalline form, it assumes a semisolid state at -65°C ; at -25°C it is a viscous oil.

The density of KB-16 is 1.3053 g/ml at 25°C ,⁴¹ the refractive index 1.4685 at 25°C ,^{23a} and the boiling point 100°C at 15 mm, 89°C at 6.5 mm, 86°C at 5.5 mm, 82°C at 4 mm, and 75°C at 2 mm.⁴³

The volatility of KB-16 is 0.87 mg/l at 25°C , slightly less than the corresponding value of 0.96 mg/l for *bis*(β -chloroethyl) sulfide (H). Several determinations of the volatility (or vapor pressure) as a function of temperature have been made.^{2,11,53} The vapor pressure at temperatures in the range of interest for chemical warfare is given by the following equation:¹¹

$$\log p \text{ (mm Hg)} = 8.91282 - \frac{2959.3}{T}$$

The standard free energy of formation of KB-16 and several thermodynamic constants of the intermediate carbamate have been calculated from the results of a series of calorimetric and equilibrium studies.⁷

8.2.3 Chemical Properties

KB-16 decomposes within 48 hours in water or in aqueous bicarbonate solutions.³ In the former case about 40 per cent of the nitrogen appears as nitric acid, the remainder disappearing from the reaction mixture. Only 5 per cent of the chlorine appears as chloride ion. In bicarbonate solution more than 90 per cent of the nitrogen is lost, presumably as nitrogen gas, and carbon dioxide and methanol are produced. About 30 per cent of the chlorine appears as chloride ion; the remainder is bound to carbon, presumably in the form of ethylene chlorohydrin. The production of chloride ion at $\text{pH } 8$ is not significantly altered in the presence of substances which react with the β -chloroethyl groups of the sulfur and nitrogen mustards. The decomposition of ethyl N-(β -chloroethyl)-N-nitrosocarbamate in aqueous solutions is similar to that of KB-16.³

One of the most characteristic reactions of KB-16 is its rapid and complete decomposition with evolution of nitrogen when treated with alcoholic ammonia or primary aliphatic amines.³ Solutions of ammonia or ethanolamine in ethylene glycol have therefore been recommended as personal or laboratory decontaminants.² However, the distinct possibility that substances of the nitrogen mustard type may be formed by this reaction should be considered in the choice of a decontaminant (see below). Secondary amines and primary aromatic amines react relatively slowly with KB-16.

In aqueous solutions, the reaction with ammonia and with primary amines is slower, perhaps because of the low solubility of the nitrosocarbamate. At $\text{pH } 8$, the main reaction with primary amino groups is carboalkoxylation, as has been demonstrated by the isolation of methyl and ethyl N-benzylcarbamates as products of the reaction of benzylamine with methyl and ethyl N-(β -chloroethyl)-N-nitrosocarbamates, respectively.³ Secondary amines (diethanolamine) are also carboalkoxylated.

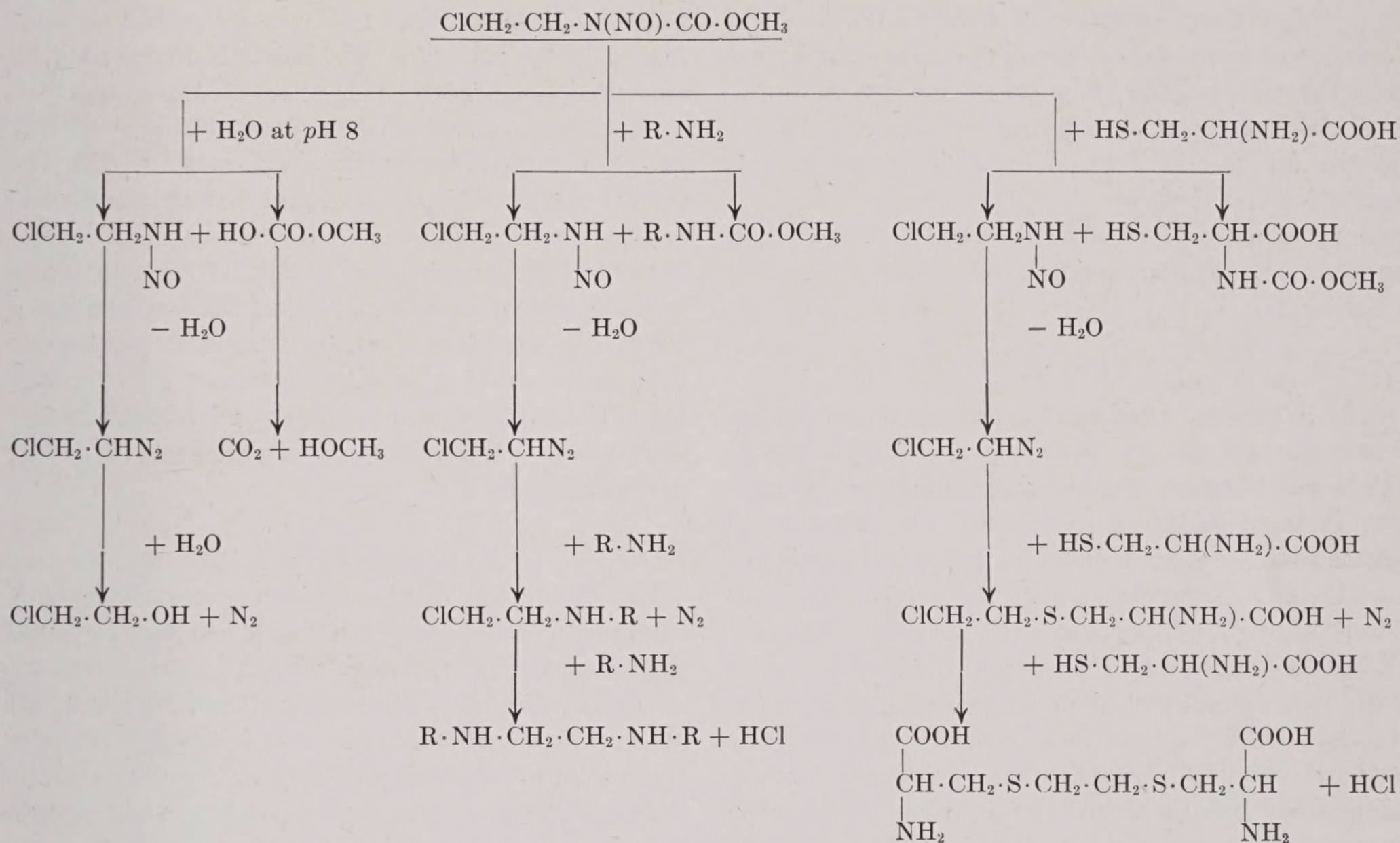
In ethereal solution, reaction with benzylamine leads to methyl N-benzylcarbamate and N,N'-dibenzylethylenediamine, the latter probably arising through benzyl- β -chloroethylamine as an intermediate.³

The amino groups of α -amino acids also react with KB-16, but the reaction is more sluggish than those of primary aliphatic amines and does not appear to go to completion. With cysteine, the reaction proceeds along several lines; both amino and sulfhydryl groups disappear. *bis*-S-(Cysteinyl)ethane, probably arising through the intermediate S-(β -chloroethyl)-cysteine, has been isolated as a product of this reaction.³ Nitrosomethylurethane also carbethoxylates the amino group of cysteine, but is far less active toward the sulfhydryl group than is KB-16.³

In solutions containing egg albumin KB-16 reacts

β -chloroethyl group into amines and into the sulfhydryl compounds. The intermediate products are of the sulfur and nitrogen mustard type, and undergo further reactions characteristic of these substances (see Chapter 19).

With regard to loci of action in tissues, it may be noted that reactions of the sulfur and nitrogen mustards involve a process of thermal solvolytic activation in water (see Chapter 20). On the other hand, the alkylation of benzylamine by KB-16 in ether solution demonstrates that this agent need not be so activated. As a result, it is possible that KB-16 can



slowly with the liberation of 1 mole of nitrogen per mole of nitrosocarbamate but no decrease in the amino nitrogen content of the protein occurs.^{3,25} The reaction with hemoglobin is rapid²⁵ and in this case some amino groups disappear, possibly by carbomethoxylation, although it has not been possible to ascertain the mode of reaction.³

The following scheme of reaction, reminiscent of those of Klobbie⁵⁸ and V. Pechmann⁵⁹ for the breakdown of nitrosomethyl- and nitrosoethylurethanes, has been proposed to explain many of the observed reactions of KB-16.³ Thus KB-16 can behave as a chloroalkylating agent, introducing the

react in fatty phases of tissues, whereas reactions of the nitrogen mustards in these loci are not equally probable.³

8.2.4 Detection and Analysis

KB-16 reacts with the DB-3 reagent to produce the characteristic blue color. Samples as small as 25 μg may be detected by use of the DB-3 tube of the United States Army M-9 Detector Kit according to the standard procedure. By heating to 200 C, the sensitivity of the tube can be increased sufficiently to permit the recognition of 1 μg .²⁶ In the absence of a guard tube, the spotted dick test of the British

Vapor Detector Kit gives an overall blue color.⁴¹ Acidified iodoplatinate paper is bleached by the vapor of the agent. Other procedures for detection involve the use of the diethylamine and diphenylbenzidine reagents or the Liebermann reaction. Positive reactions given by the decomposition products of KB-16 limit the usefulness of these methods.

The most useful method for the analysis of KB-16 depends upon the quantitative evolution of nitrogen which occurs when the compound is treated with primary amines^{16i,23e} or with alcoholic alkali.⁴¹ This method is suitable for use as an assay method or for analysis of samples collected in chamber or field tests, and has the advantage of specificity to the extent that it measures only the nitrosated material. The Griess reagent as used for nitrites can be employed for the field or chamber analysis of this agent.⁴³

A more detailed discussion of the detection and analysis of the nitrosocarbamates will be found in Chapter 34.

8.2.5 Stability

KB-16 and its homologous esters are thermally unstable. Decomposition with gas evolution occurs at rates which make storage impractical.^{2,41} In steel containers with 25 per cent void, the pressure increase per day is appreciable at temperatures as low as 4 C and amounts to about 2 psi at room temperature.² At high temperatures the decomposition becomes even more rapid, the pressure increase in glass with 50 per cent void amounting to about 4.5 psi per day at 60 C.⁴¹ No significant difference between the rates of pressure development in glass and steel containers has been observed (unpublished data), even though steel appears to be attacked.⁴¹ The purity of the sample has a considerable effect on the rate of thermal decomposition, carefully purified material decomposing at a lower rate than crude material.^{23d} The stability of preparations made by nitrosation with nitrous gases is as good as or better than that of flash-distilled samples. Decomposition is accelerated by acidic and phenolic substances and by zinc and magnesium oxides.^{2,41} There is disagreement as to the effect of traces of water, weak bases, or contact with metals other than steel.^{2,41}

The gas produced during the decomposition of KB-16 consists principally of nitrogen; oxides of nitrogen, carbon dioxide, and hydrogen chloride have also been identified.^{16h,23c,41}

In spite of intensive searches for a stabilizer to prevent or minimize the spontaneous decomposition of KB-16, little success has been achieved. Few of the tested substances were of any value and none produced a pronounced increase in storage stability. The tested compounds include organic and inorganic bases, acids and derivatives of acids, hydroxy and mercaptan derivatives, oxidizing agents, inert liquids and solids, salts and complexes of heavy metals, and numerous miscellaneous compounds.^{23c,41}

Few reliable data are available on the stability of KB-16 to detonation in munitions.^{16m,30,31,41} The results of a field trial with 105-mm shell supply no definitive information.³⁰ In a small chamber, detonation of a 75-mm shell charged KB-16 resulted in more or less complete destruction of the agent;³¹ in similar tests ethyl-*bis*(β -chloroethyl)amine (HN1) was also destroyed but *tris*(β -chloroethyl)amine (HN3) was not. It may be noted that the conditions of these tests were more severe than would be encountered in the field, and that HN1 can effectively be dispersed from M47A2 bombs.²⁸ KB-16 is not destroyed to any great extent by the milder explosions that occur when it is dispersed from glass bottles either in the field by means of a standard detonator³¹ or in a 2-cu m chamber by means of a blasting cap or detonator.^{16m,31}

8.2.6 Decontamination

Rapid surveys of the reactions of KB-16, with emphasis on reactions of possible use in decontamination, have been carried out both in this country and in Great Britain.^{20,47} The reagents examined included bleaching powder, chloramides, a number of inorganic salts in solution, mineral and organic bases, at least one strong oxidizing agent, and reducing agents. Solid bleach or lime slurry would appear to be suitable for field use. Caustic soda or alcoholic ammonia has been recommended for laboratory use, and aqueous ethanolamine for personal use. In line with these recommendations, groups concerned with the synthesis of the agent have used solutions of ammonia or ethanolamine in alcohol or ethylene glycol for personal, laboratory, and pilot plant decontamination.^{2,23a} As stated above, ammonia or primary amines should be used with caution because of the possibility of producing toxic intermediates.³

For treatment of eyes contaminated with KB-16, mild alkalies and reducing agents (e.g., BAL) should be more effective than in the case of nitrogen mustards.^{25,47}

8.2.7

Protection

The canisters of modern gas masks afford adequate protection against the vapor KB-16.^{19,41} For details the reader is also referred to the Summary Technical Report of NDRC Division 10.

The chloramide impregnation of clothing would appear to offer little resistance to KB-16, because this agent fails to react with chloramine-T or with

various impregnites.²⁰ It may be assumed that clothing containing activated carbon would effectively exclude the vapor of the agent.

8.3 CHEMICAL STRUCTURE IN RELATION TO TOXICITY

In Table 2 are presented data on the toxicity for mice of compounds in which the N-nitroso, N-(β-

TABLE 2. Toxicities of N-substituted aliphatic carbamates for mice.

Most of the data are taken from reference 10. The mice were observed for 10–15 days after exposure for 10 minutes to the stated nominal concentration. In the case of one compound, methyl N-methyl-N-nitrosocarbamate, the data were obtained from reference 48 and relate to rats exposed for 30 minutes.

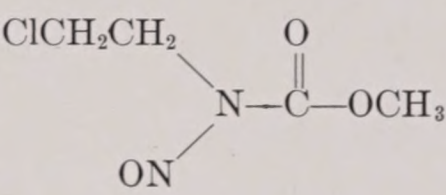
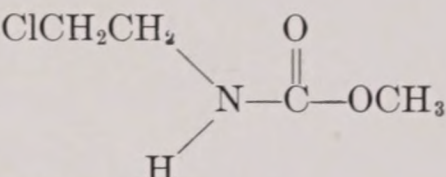
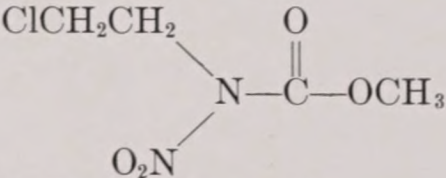
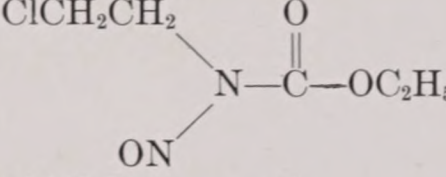
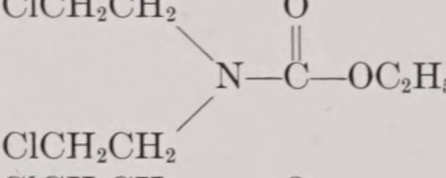
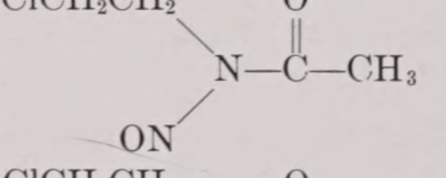
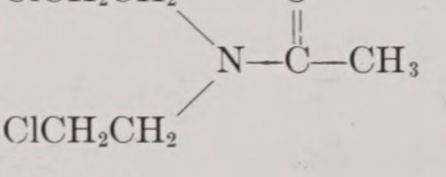
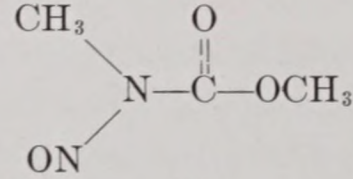
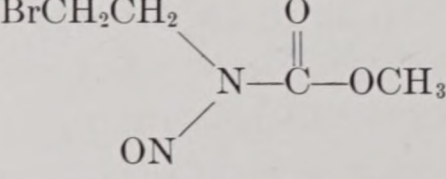
Compound	Structural formula	Nominal conc. (mg/l)	Mortality for 10-min exposure
<i>The prototype compound</i>			
Methyl N-(β-chloroethyl)-N-nitrosocarbamate (KB-16)		0.036	LC ₅₀
<i>Effect of replacement of the N-nitroso group</i>			
Methyl N-(β-chloroethyl)carbamate		1.0	0/20
Methyl N-(β-chloroethyl)-N-nitrosocarbamate		1.3	0/20
Ethyl N-(β-chloroethyl)-N-nitrosocarbamate		0.075	LC ₅₀
Ethyl N,N-bis(β-chloroethyl)carbamate		0.4	0/40
N-(β-chloroethyl)-N-nitrosoacetamide		0.046	LC ₅₀
N,N-bis(β-chloroethyl)acetamide		0.5	0/10
<i>Effect of replacement of the N-(β-chloroethyl) group</i>			
Methyl N-methyl-N-nitrosocarbamate		0.26 (30 min) 0.13 (30 min)	1/4 (rats) 3/4 (rats)
Methyl N-(β-bromoethyl)-N-nitrosocarbamate		0.2 0.82	0/10 10/20

TABLE 2 (Continued).

Compound	Structural formula	Nominal conc. (mg/l)	Mortality for 10-min exposure
Methyl N-(β -hydroxyethyl)-N-nitrosocarbamate	$\begin{array}{c} \text{HOCH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}_3 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.3	0/20
Methyl N-(β -chloropropyl)-N-nitrosocarbamate	$\begin{array}{c} \text{CH}_3\text{CHClCH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}_3 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.3	0/10
Methyl N-butyl-N-nitrosocarbamate	$\begin{array}{c} \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}_3 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.3	1/20
Methyl N-phenethyl-N-nitrosocarbamate	$\begin{array}{c} \text{C}_6\text{H}_5-\text{CH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}_3 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.97	0/20
<i>Effect of replacement of the methoxy group</i>			
Ethyl N- β -chloroethyl-N-nitrosocarbamate	$\begin{array}{c} \text{ClCH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}_2\text{CH}_3 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.075	LC_{50}
β -Fluoroethyl N-(β -chloroethyl)-N-nitrosocarbamate	$\begin{array}{c} \text{ClCH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}_2\text{CH}_2\text{F} \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.1	11/15
		0.2	11/20
		0.5	10/20
Isopropyl N-(β -chloroethyl)-N-nitrosocarbamate	$\begin{array}{c} \text{ClCH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OCH}(\text{CH}_3)_2 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.1	6/20
		0.12	20/20
		0.2	16/18
Butyl N-(β -chloroethyl)-N-nitrosocarbamate	$\begin{array}{c} \text{ClCH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{OC}_4\text{H}_9 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.16	LC_{50}
		0.44	LC_{50}
N-(β -chloroethyl)-N-nitrosoacetamide	$\begin{array}{c} \text{ClCH}_2\text{CH}_2 \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad \text{N}-\text{C}-\text{CH}_3 \\ \quad \quad \quad \diagup \\ \quad \quad \quad \text{ON} \end{array}$	0.046	LC_{50}

chloroethyl), and methoxy groups of KB-16 are replaced by other substituents. On the basis of these data, and subject to their limitations, the following conclusions can be drawn.

1. The N-nitroso group is essential for high toxicity. Its replacement by another group has always resulted in at least a 30-fold reduction in toxic potency.

2. The N-(β -chloroethyl) group is essential for highest toxicity, but moderate toxicity is possessed by some compounds in which it is replaced by an alkyl group (e.g., ethyl N-methyl-N-nitrosocarbamate apparently possesses one-tenth the potency of ethyl N-(β -chloroethyl)-N-nitrosocarbamate).

3. The methoxy group, although optimal, is not essential for high toxicity. Its replacement by a methyl group (to form N-(β -chloroethyl)-N-nitrosoacetamide) results in an insignificant decrease in potency, and the ethoxy analog is about one-half as toxic as KB-16.

Toxicity data for other species, vesicancy tests, and determinations of eye-injuring potency are not sufficiently complete to permit analyses of the relative potencies of members of this series. Such data as are available indicate the relative superiority of KB-16 and are not inconsistent with the other conclusions drawn from the toxicity tests with mice.

Compounds which possess a fluoroacetate-like toxicity by virtue of the presence of an $\text{FCH}_2\text{C}(=\text{O})\text{—}$ group are an exception to this generalization (see Chapter 10).

8.4 TOXICOLOGY

Of the following toxicological sections, those on detectability by odor and sensory irritation, vesicancy, and eye-injuring action bear most directly on the evaluation of KB-16 and related compounds as chemical warfare agents.

8.4.1 Detectability by Odor and Sensory Irritation

The vapor of KB-16 has a pleasant odor, sometimes described as sweet or fruity, which can be detected by smell only at concentrations several times greater than those required for H. Men exposed to relatively high concentrations (i.e., 70 $\mu\text{g}/\text{l}$ nominal) for 30 seconds detect the odor but experience no sensory irritation,⁴¹ and concentrations as high as 0.2–0.4 mg/l elicit no signs of irritation in animals.^{16b} These properties of KB-16 vapor, considered in relation to its eye-injuring potency and the delayed onset of the injuries caused by casualty-producing dosages (see the following section), confer upon it some insidiousness. However, in this regard it is not notably superior to some of the nitrogen mustards (e.g., HN3), which are probably less easily detected by odor and not notably inferior in eye-injuring potency (see Chapter 6).

Laboratory determinations of the median detectable concentrations in $\mu\text{g}/\text{l}$ of KB-16, H, ethyl-bis(β -chloroethyl)amine (HN1), and HN3 are tabulated below. Attention is directed primarily to the *relative* values, inasmuch as the absolute values are not necessarily of significance for field conditions.

Agent	$\mu\text{g}/\text{l}$	Reference
H		
Plant run	Levinstein	0.6 34
Pure	thiodiglycol	1.8 35
KB-16	7 $\pm$	16 1, 41
HN1		
Plant run	13	34
Pure	17	33
HN3		
Plant run	15 $\pm$	37

The vapor of N-(β -chloroethyl)-N-nitrosoacetamide does not possess the insidiousness of KB-16.^{16f,17j}

8.4.2 Toxicity

INHALATION TOXICITY

In Table 3 data on the toxicity of KB-16 vapor

TABLE 3. Inhalation toxicity of KB-16 in comparison with mustard gas (H) and tris(β -chloroethyl)amine (HN3).

Species	Approximate nominal LC_{50} in mg/l for 10-min exposure and 15-day observation period.*		
	KB-16	H	HN3
Mouse	0.036 (259) ^{†4,16g}	0.12 ^{1,16l,q}	0.12 ^{§16i}
Rat	0.1–0.2 (19) ^{16b} 0.035 (60) ⁴	0.1 ^{16l,p}	0.2 ^{–16g,o,22d}
Guinea pig	0.2 $\pm$ (17) ^{16b}	0.2 ^{–16l}	>0.2 ^{16g}
Rabbit	>0.2 (7) ^{4,16b,41}	0.28 ^{†38}	0.14 ^{16s}
Cat	0.1–0.2 (11) ^{16b,j}	0.07 ^{16l}	0.08 ^{16k,o}
Dog	0.1 (14) ^{16b,i,17d}	0.07 ^{16l}	0.1 ^{16k,o,55}
Goat	0.3 (6) ^{17d}	0.19 ^{†36}	
Monkey	0.2–0.5 (6) ^{17d}	0.08 ^{16p}	

* In the case of KB-16 some deaths occurred among the larger species after observation periods as long as 15–30 days and were included in estimating the LC_{50} 's.

† The figures in parenthesis give the number of animals upon which the estimated LC_{50} 's are based.

‡ Analytically determined concentration.

§ The analytical LC_{50} is about 0.055 mg/l.

for various animal species are set forth in comparison with corresponding data for H and HN3. One of the early observations arousing interest in KB-16 was the discovery that for mice it is several times more toxic than H. When tests were made with other species, however, no such differential in favor of KB-16 was found, except possibly in the case of the rat. Indeed, it may be questioned whether KB-16 is as toxic as H for the larger mammalian species which have been studied.

The only evidence bearing on the relation of toxicity of KB-16 to exposure time is the demonstration that the $L(Ct)_{50}$ for mice is approximately the same for 30-minute exposures as for 10-minute exposures,^{16b} and the result of a single experiment in which a dog succumbed 23 days after exposure to a total vapor dosage of approximately 1,100 mg min/m³ administered during three 8-hour periods on successive days.^{17d}

During exposure to KB-16 vapor at concentrations as high as 0.2–0.4 mg/l, animals exhibit no signs of discomfort or irritation.^{16b} Concentrations considerably in excess of the 10-minute LC_{50} 's occasionally caused closing of the eyes, but the irritation was mild and not accompanied by profuse lachrimation.

The development of symptoms after gassing with KB-16 is usually delayed for 12–24 hours and follows the same general pattern in different species.^{4,16b,17d} Respiratory distress becomes prominent. The animals appear depressed and stop taking food and water; as a consequence weight loss may be precipi-

tous. Severe eye injuries also develop (see Section 8.4.4). In nonfatal cases the symptoms slowly subside. In fatal cases respiration may become labored and terminate in death after 3–10 days, or the animals may slowly waste away and die as late as 3–4 weeks after exposure.

The principal pathological changes occurring in animals gassed with KB-16 are found in the eyes (see Section 8.4.4) and the respiratory tract.^{4,16b,11,17g} In mice the most severe changes are confined to the nasal and nasolaryngeal mucosa, and an exudate, first fluid and later mucopurulent, is often produced in sufficient amount to block the air passages. The trachea and bronchi show much less damage. The lungs may become hyperemic but pulmonary edema is minimal and pneumonia does not ordinarily develop. In larger species, perhaps because the larger size of the air passages permits further penetration of the vapor, nasal injury is accompanied by severe pathological changes in the deeper parts of the respiratory system. The larynx, trachea, and bronchi are severely involved, and pulmonary injury with consolidation occurs. The pneumonia often appears in focal patches around the bronchi. Degenerative material cast off from the larger respiratory tubes often blocks some of the larger and smaller bronchial passages.

In general the bone marrow is little affected.^{4,6} Atrophy of the lymphoid organs with rhexis of the lymphocytes of the thymus gland and the splenic follicles has been reported in some species^{17g} but was not found to be conspicuous in another investigation.⁴ Hematological studies fail to reveal the conspicuous changes in numbers of circulating white blood cells which characterize severe intoxication with the sulfur and nitrogen mustards, although a rise followed by a fall in lymphocyte count has been reported in mice exposed to about eight $L(Ct)_{50}$ dosages of KB-16 vapor.^{17g} In some instances limited degenerative changes, possibly secondary to respiratory embarrassment, have been observed in the liver and kidney. In mice and rats the digestive tract from upper esophagus to anus is often markedly distended with gas but no hemorrhages or perforations have been observed;⁴ the distention is probably caused by swallowing of air, which occurs because of mouth breathing and difficulty in respiration.

The available data suggest that the principal pathological effects of ethyl N-(β -chloroethyl)-N-nitrosocarbamate and of N-(β -chloroethyl)-N-nitrosoacetamide are similar to those of KB-16.^{6,16f,g,17j}

TOXICITY THROUGH THE SKIN

In its actions on and through the skin, KB-16 is relatively ineffective as a lethal agent when compared with H, HN1, or HN3. In spite of the high sensitivity of mice to the inhaled vapor, exposure of only the bodies of animals of this species to KB-16 vapor at a nominal dosage of 6,900 mg min/m³ ($t = 10$ min) caused no deaths within 15 days; 11,300 mg min/m³ killed 1/5 unshaved mice; and 13,000 mg min/m³ killed 6/6 mice with shaved backs.^{16b} For other agents approximate nominal $L(Ct)_{50}$'s ($t = 10$ min) for mice upon body only exposure are: H, 4,000 mg min/m³; HN1, 5,000 mg min/m³; HN3, 2,000 mg min/m³ (analytical value = 1,000); and L, 2,100 mg min/m³.¹³ The toxicity of liquid KB-16 applied to the shaved skin of mice is also low when inhalation of vapor is minimized. Necrosis of the skin and ulcer formation occur at the site of application but a *minimum* value for the LD_{50} is believed to be 62 mg/kg.⁴ Corresponding values for other agents are: H, 92 mg/kg; HN1, 13 mg/kg; HN3, 7–20 mg/kg (see Chapter 22). It may be noted that all of these values are high in comparison with the percutaneous LD_{50} 's for some of the compounds considered in Chapter 9.

TOXICITY BY INJECTION

Parenteral injections, although they have no direct bearing on chemical warfare, supply useful information concerning the toxicological properties of KB-16. LD_{50} 's upon intravenous injection are: mouse, 0.45 mg/kg; rat, 1.1 mg/kg; and rabbit, approximately 2.0 mg/kg.⁴ The subcutaneous LD_{50} for the mouse is 9.0 mg/kg;⁴ and those for the rat and rabbit approximately 8 and 20 mg/kg, respectively.⁴¹ Even large doses are without immediate pharmacological effects, and subsequent developments reveal no neurological injury, central nervous or gastrointestinal action, pronounced leucopenic action, or significant changes in the total number of circulating white blood cells.⁴ The conspicuous pathological changes occur in the lungs, which become distended, moist, and hyperemic. They sink in water and on cutting a pinkish, foamy fluid runs from the lungs and trachea. A small amount of pleural fluid accumulates. The heart is often dilated but gross pathological changes elsewhere are conspicuously absent. Venous congestion of the liver and myocardial injury with focal necroses are occasionally but not constantly observed. The thymus gland and spleen are usually but not markedly decreased in size — probably the re-

sult of a nonspecific lymphoid involution. In some instances (e.g., in rabbits receiving large doses) there is evidence of lymphocytic fragmentation in the spleen, lymph nodes, and thymus. The bone marrow usually appears normal, although evidence of leucoblastic stimulation appears in some rabbits.⁴ It has been reported that one of two dogs receiving 18 mg/kg intravenously died in 4 days with aplasia of the bone marrow and drastic leucopenia, involving both lymphocytes and granulocytes.⁶ It has been concluded that intravenously injected KB-16 causes death by producing fatal pulmonary edema, which develops slowly over a period of 2–8 days.^{4,5}

Injections by various routes demonstrate that KB-16 reacts with liberation of gas (presumably N₂) in the first capillary bed it reaches.⁴ Circulatory stasis may occur, in some cases possibly because of vessel spasm or thrombosis, so that contact with the tissue may be prolonged. These observations give an explanation for the finding that the principal pathological changes following inhalation or intravenous injection occur in the lungs. The liberation of gas, which occurs in the tissues to which injected KB-16 is first carried and which also occurs when KB-16 is added to tissue suspensions, undoubtedly contributes ischemic injury to the chemical injury produced by the direct reactions of KB-16 with tissue components. The liberation of gas following inhalation of KB-16 vapor is presumably not sufficient to be significant.

TOXICITY BY MOUTH

KB-16 is moderately toxic when administered by stomach tube. The *LD*₅₀'s are in the order of 20 mg/kg for the rat and 15 mg/kg for the rabbit.⁴¹ The substance is immediately irritating, as evidenced by vomiting in dogs, and it produces severe esophageal and gastric damage.^{17g} In the rat, vesicles similar to those produced by lewisite oxide have been found in the stomach at autopsy.⁴¹ Lung pathology has been observed in some cases^{17g} and the absorption of KB-16 from the gastrointestinal tract has been demonstrated by the appearance of gas bubbles in the hepatic portal vein.⁴ Death occurs after from one-half day to many days and is preceded by pronounced weight loss when survival is prolonged.^{17g,41}

That KB-16 presents some hazards as a water contaminant is demonstrated by the virtually 100 per cent mortality of mice, rats, and dogs whose supply of drinking water was contaminated with 0.5–1.0 mg/ml.^{17e} Only few deaths occurred when the water

was contaminated with 0.1 mg/ml. In most of the experiments the contaminated water was freshly prepared each day. In spite of the fact that aqueous solutions of KB-16 decompose within 48 hours (see Section 8.2.3), mice whose drinking water was contaminated with 1.0 mg/ml of KB-16 at the beginning of one experiment died almost as quickly as those whose contaminated water supply was freshly prepared at daily intervals.

8.4.3

Vesicant Action

In comparison with H, the vesicancy of KB-16 is of a low order^{14,41,46} and screening tests indicate that none of the related compounds is more potent.¹⁴ A direct comparison of "absolute vesicancies," determined by application of agents diluted with benzene and covered to prevent evaporation, reveals that KB-16 is about $\frac{1}{3}$ to $\frac{1}{4}$ as potent as H.⁴¹ As shown in Table 4, small doses of liquid KB-16 applied

TABLE 4. Vesicancy of KB-16.¹⁴

For the sake of comparison, data obtained with H and L are included. All applications of the vesicants were made during winter weather (January 1943) to the skin of the forearms of human subjects at room temperatures of 63–72 F and relative humidities of 14–37 per cent.

Agent	Dose (μ g)	Days after applica- tion	Erythemas		Blisters	
KB-16	200	2	7/28	(4 mm)	0/28	...
		7	7/9	...	1/9	(2 mm)
H	65	2	112/119	(7 mm)	70/119	(5 mm)
L	95	2	285/290	(8+mm)	279/290	(6 mm)

to the skin in the usual way (i.e., undiluted and with evaporation permitted) produce far less injury than do H or L. Tested more realistically in relatively large doses (drops 1.1 mm in diameter), it produces injuries which after 3 days are no more severe than those elicited by HN3 or HN2.⁴⁶ It is known from other data (see Chapter 6) that, under the moderate conditions of temperature and humidity prevailing in the above test, these nitrogen mustards are no more than one-fourth as vesicant as H. All observations^{14,41,46,52a} indicate that skin injuries due to KB-16 require considerably longer (i.e., 5–6 days) to attain maximum severity than do those usually produced under similar conditions by H, L, or the nitrogen mustards.

It should be noted that all of the above observations were confined to applications of the liquid agent to the not visibly sweating skin of physically inactive subjects at moderate temperatures and humidities.

No determinations have been made of the vesicant potency of liquid KB-16 on hot, sweating skin, of the vapor under any conditions, or of the effectiveness of either the liquid or the vapor through ordinary or protective clothing.

8.4.4 Eye-Injuring Action

Numerous observations on the effect of KB-16 vapor on human and animal eyes demonstrate that the agent is an insidious and potent eye-injuring agent.^{16a,b,c,g,h,i,k,17a,d,e,f,i,j,24a,26,32,44,52a,b} As has been mentioned, no exposure symptoms are produced in animals by even high concentrations (i.e., 0.2–0.4 mg/l), and exposures entirely undetected have sufficed to produce moderately severe injuries in laboratory workers.^{52a} The onset of injury and accompanying symptoms is more delayed than in the case of H, and much more delayed than in the case of the arsenicals. There is an asymptomatic latent period of many hours. Maximal damage develops after from two to several days, and recovery is protracted. Corneal edema, opacity, and delayed but extensive vascularization are the most prominent symptoms. The conjunctivas are also injured, although less extensively than in the case of H. Iritis occurs but is not so conspicuous as in eyes exposed to HN2 or H. Delayed relapses such as occur in the case of H have not been observed.

An interesting preliminary report^{16p} indicates that, in addition to the injuries just described, severe *retinal damage* can be produced in animals by exposures to relatively small dosages of KB-16 vapor which produce only moderate conjunctivitis and slight and transient superficial keratitis. Changes in the retinas of cats examined 3–14 days after exposure of the animals to 0.05 mg/l for 10 minutes consisted of: (1) slight increases in glial cells and perivascular macrophages, with hyperchromaticity of ganglion cells; (2) restricted zones of perivascular cuffing with leucocytes, resembling a periarteritis; and (3) intense chorioretinitis with subhyaloid hemorrhages, migration and phagocytoses of pigment, and *extensive chromatolysis and destruction of ganglion cells*. Comparable exposures to HN1 produced no morphological changes in the retinal ganglion cells, although clusters of leucocytes adhering to the endothelium of the blood vessels represented a difference from the normal retina. Exposures to H vapor (0.04 mg/l for 10 minutes) likewise produced the clustering of leucocytes, and in addition isolated small patches of chorioretinitis; however, changes in the neural elements

were absent or at most mild compared with those produced by KB-16 at the slightly higher dosage.

Data to be summarized in the following paragraphs lead to the conclusion that KB-16 vapor is a distinctly more insidious eye-injuring agent than H vapor but not necessarily a more potent injurant when assessed on a dosage (*Ct*) basis. In this respect KB-16 is similar to HN3 (see Chapter 6).

EXPOSURE OF HUMAN EYES TO SMALL DOSAGES OF KB-16 VAPOR

The eye injuries produced by the vapor of KB-16 at small and minimal dosages may best be illustrated by citation of the case histories of accidentally exposed laboratory workers.

In one case^{52a} some KB-16 was splashed on the left side of the face. It was immediately decontaminated and the liquid presumably did not enter the eye, as the worker was wearing glasses. Nevertheless as a precaution the eyes were quickly washed with water. There were no ocular symptoms on the day of the accident. On the following day both eyes were slightly sore but normal duties could be carried out. Ophthalmic examinations 3–22 days after the accident revealed the following effects. *3 days*. The conjunctivas were hyperemic, the injection being more marked in the palpebral aperture than elsewhere. The cornea did not stain with fluorescein but scattered epithelial cells showed hydropic degeneration. The pupils were normal and there was no iritis. *4 days*. The eyes were more uncomfortable and the patient experienced slight difficulty in keeping them open. The conjunctivas were more hyperemic and there was epithelial bedewing all across the palpebral aperture. All the limbal blood vessels were congested. The substantia propria of the cornea was normal. The lids were slightly swollen. There was no chemosis and no iritis. *5 days*. The symptoms were worse and an attack of blepharospasm and photophobia occurred. Subepithelial cellular infiltrates could be seen in the left eye. *6 days*. The congestion was worse and the left cornea still bedewed. *7 days*. The patient felt that his sight was worse. Visual acuity was reduced from 6/12 (on the fourth day) to 6/24. The congestion was more marked and the superficial layers of the substantia propria were densely infiltrated with cells but not edematous. *9 days*. The lids were slightly sticky and puffy, the conjunctivas very injected. The interior of the eyes was normal. *11 days*. The limbal loops showed great activity and appeared to be advancing on to the corneas from all meridians. *16 days*. The right eye was slightly better, the left showed further roughening of the epithelium and slight edema of the substantia propria. The limbal loops were advancing. *20 days*. Photophobia persisted and the eyes appeared worse. Conjunctival injection was marked. The margins of both corneas were invaded with a rich superficial vascular net. The corneas were full of cells at all levels. *22 days*. The limbal loops were still extending. The symptoms were somewhat alleviated but definite objective improvement had not started. *Comment*. The main points of interest are the absence of immediate symptoms, the long latent period, and the delayed recovery, even though the dose was insufficient to produce pupillary contraction or iritis. The prognosis was

considered good in view of the course of the case next to be described.

In a second case ^{52a} a chemist had been working with KB-16 for 3 days during which time he smelled nothing and experienced no sensation to suggest that he was being exposed to the vapor. On the second day his eyes were slightly bloodshot but not painful. On the evening of the third day of work he had a severe headache and pain in the eyes, and on awakening during the night found himself unable to keep his eyes open. He was examined at 3-22 days after he commenced his work. *3 days.* The lids were only slightly swollen but the patient was unable to keep his eyes open. There was lacrimation but no discharge. The conjunctivas were not very congested and there were no hemorrhages. In the palpebral aperture there was a band of epithelial edema and punctate staining. The pupils were small and their reaction to light poor. The patient had nasal discharge. *4 days.* The eyes were still closed and the pain, now a gritty feeling, was relieved by phenacetin. The corneas appeared improved. The lids were slightly reddened. *5 days.* The gritty feeling persisted. There were no signs of iritis but the limbal loops were beginning to encroach on the corneas. *6 days.* The eyes were much better and could be kept open for periods of an hour or more, with attacks of blepharospasm between. The conjunctival injection was almost limited to the palpebral apertures. The epithelium was bedewed but an ocular infiltration was beginning under Bowman's membrane. The deep structures were normal. *7 days.* The eyes could be kept open much better but lacrimation persisted. There were infiltrates throughout the substantia propria. *9 days.* Photophobia persisted and there was a slight whitish discharge. The epithelial bedewing in the palpebral apertures was less pronounced. The conjunctivas were still slightly injected. *11 days.* The eyes were about as above except that new vessels were extending in open loops onto the corneas. Photophobia persisted. *15 days.* The newly formed superficial vessels on the cornea were beginning to empty. *20 days.* The photophobia had practically disappeared and the eyes were nearly normal on macroscopic examination. *22 days.* The eyes were practically symptomless. The limbal loops had extended onto the cornea all around in both eyes but were mostly empty and disappearing. Subepithelial infiltrating cells were fewer. A few hydropic cells remained in a line on the palpebral fissure of one eye. *Comment.* The main points are that the exposure was unsuspected and symptoms delayed for 2 days. They then became severe. Virtually complete recovery had occurred within 22 days.

A number of investigators working with KB-16 and presumably receiving minimal vapor dosages have developed mild ocular changes.^{16c} The conjunctivas showed at most only mild congestion. Examination of the corneas revealed superficial punctate nebulae largely peripheral in location and almost always confined to the interpalpebral area. The nebulae sometimes escaped detection on slit lamp examination but were seen after fluorescein staining. Clinical notes also mention a Stahli's line, unduly prominent corneal nerves but no alteration in corneal sensibility, and fine punctate hyaline areas best seen

with lateral illumination or retroillumination and disappearing within 2-3 weeks. The observed changes were of a type commonly observed in various non-specific irritations of the eye and are difficult to evaluate. They were insufficient to produce significant subjective symptoms or loss of visual acuity. Nevertheless, in view of the slow development of the pathological changes caused by KB-16 vapor, it has been recommended that individuals showing such lesions avoid any possibility of further exposures for 1-2 weeks.²⁶

COMPARISON OF KB-16 WITH OTHER AGENTS ON BASIS OF EFFECTS OF RELATIVELY LARGE VAPOR DOSAGES ON ANIMAL EYES

Quantitative comparisons of the potencies of different agents in terms of the dosages necessary to produce eye injuries of casualty severity are difficult at best and for KB-16 there exist no detailed quantitative studies such as have been made with H.²⁹ Of the numerous interim studies with animals, two ^{16k,24a} permit a more or less direct semiquantitative comparison between the potencies of KB-16 and H. Both studies (Tables 5, 6, and 7) indicate that the poten-

TABLE 5. Effects of the saturated vapors of KB-16 and H on the eyes of rabbits: effect of exposure time on severity of injury.^{24a}

Exposure time (sec)	Agent	
	KB-16	H
15	Mild conjunctival lesion, slight corneal lesion. Rapid recovery.	Minor conjunctival lesion. Rapid recovery.
30	Injury variable. Rare perforation, occasional complete recovery.	Injury variable. Complete recovery in most cases.
60	Mild permanent damage. Perforation in some cases.	Moderate permanent damage. No cases of perforation.
120	About same as 1-minute exposure.	Severe damage. Many cases of perforation.

cies of the two agents are of the same order of magnitude, the principal difference being that the onset of damage and possibly the rate of recovery are more delayed in the case of KB-16. In so far as this con-

TABLE 6. Effects of the saturated vapors of KB-16 and H on the eyes of rabbits: tabulation of types and relative severities of injuries.^{24a}

The eyes were protopsed and exposed for 1 minute at 22–24 C to vapor cups containing KB-16 or H. At 23 C the volatility of KB-16 is approximately 0.8 mg/l, that of H approximately 0.9 mg/l.¹¹

Characteristic of injury	Agent	
	KB-16	H
Latent period for severe injury	18–36 hr	6–16 hr
Conjunctival reaction:	+	+++
Redness	+++	++
Chemosis	+	+++
Hemorrhagic necrosis	0	++
Ischemic necrosis	0	+++
Corneal reaction:	+++	++
Edema	+++	++
Vascularization	++++	++
Ulceration	+	++
Residual opacity	+	++
Purulent discharge	+	++
Iritis	+	++
Relapse	0?	++

clusion may be extrapolated to man,^b it may be concluded, as with H, that 50 mg min/m³ ($t < 8$ hours) of KB-16 vapor would be the maximum dosage to which unmasked troops could be exposed without danger of significant eye damage, and that 200 mg min/m³ would suffice to produce totally incapacitating eye injuries of several days' duration.³⁹

In one of the two studies mentioned above the eyes of rabbits were exposed to approximately saturated vapor of KB-16 and of H at 22–24 C for short periods, with the results summarized in Tables 5 and 6.^{24a} For each exposure time the vapor dosages of the two agents were approximately the same, inasmuch as the volatility of H is only slightly greater than that of KB-16, but the high vapor concentrations admittedly represent an artificial situation. In the other study^{16k} dogs were exposed for 10 minutes to much lower concentrations of each agent, with the results summarized in Table 7.

LIQUID CONTAMINATION OF THE EYE

As in the case of other vesicant agents, small liquid drops (i.e., 0.5 mg or less) of KB-16 produce very severe and prolonged injury, frequently leading to permanent loss of sight.^{52b} Like H and in contrast to the arsenicals, KB-16 applied in this way does not evoke a severe immediate reaction. Discharge and edema reach their height on the third day. Lesions

^b It is known for H and the nitrogen mustards that the animal (i.e., rabbit) eye is distinctly less susceptible to injury than the human eye. Whether this holds for KB-16 is not known.

TABLE 7. Effects of the vapors of KB-16 and H on the eyes of dogs.^{16k} The dogs were exposed for 10 minutes to nominal concentrations of the agents in a chamber operated at a flow rate of $\frac{1}{2}$ –1 chamber volume per minute.

Conc. (mg/l)	Agent	
	KB-16	H
0.01	No irritation during exposure. No eye damage developed.	No irritation during exposure. Possible harassment for 1–2 days due to very mild corneal swelling which developed within 24 hours.
0.02	No irritation during exposure. Minor conjunctival irritation and corneal edema with increased reluctance developed within 1 day and persisted for 8–10 days. Negligible interference with vision.	Almost no irritation during exposure. Possible harassment for 3 days due to mild corneal and conjunctival symptoms.
0.04	No irritation during exposure. Serious interference with vision for at least 4 weeks beginning at 2 days, due chiefly to corneal damage which became maximal at 5 days and subsided very slowly, leaving residual opacity.	Slight irritation during exposure. Serious interference with vision for 2–3 weeks beginning at 1 day, due to corneal changes (edema, opacity, and ulceration after 1–2 days) and inflammation of the conjunctivas and lids. Maximal damage at 4½ days. Possibly some permanent damage.

involving the lids appear to be more painful than in the case of H, and there is a greater tendency to vascularization of the cornea and to iritis but not so much tendency to delayed relapses. In comparison with HN2, the iritis produced by KB-16 is less violent and severe intraocular hemorrhages do not occur.

POTENCY OF COMPOUNDS RELATED TO KB-16

Preliminary data indicate that N-(β -chloroethyl)-N-nitrosoacetamide is approximately as potent an eye-injurer as KB-16 itself,^{16f,17j} ethyl N-(β -chloroethyl)-N-nitrosocarbamate is $\frac{1}{2}$ –1 times as potent,^{16a,17f,i} the corresponding isopropyl ester and methyl N-butyl-N-nitrosocarbamate are less than one-half as potent,¹⁷ⁱ and ethyl N-methyl-N-nitrosocarbamate is no more than one-tenth as potent.^{17e,i}

8.4.5 Physiological Mechanism

The toxicological data summarized above demonstrate that the action of KB-16 is confined to a fairly severe local necrotizing action on tissues with which

it comes in contact. Practically speaking these tissues are those of the eye, respiratory tract, and, when the agent is ingested, the gut. In large, intravenously administered doses the agent lacks the gross pharmacological actions which characterize H and the nitrogen mustards (Chapter 22).

The chemical properties which presumably underlie the necrotizing action of KB-16 and the less toxic related compounds were reviewed above (Section 8.2.3). In résumé two types of reaction have been demonstrated to occur with substances of biological interest in aqueous solutions at *pH* 8. First, a general property of N-alkyl-N-nitrosocarbamic acid esters is the capacity to transform RNH_2 groups into $\text{R}\cdot\text{NH}\cdot\text{CO}\cdot\text{OR}'$ groups (carbomethoxylation, carbethoxylation, etc.). This reaction characterizes not only KB-16 and the corresponding ethyl ester, but also nitrosocarbamates (i.e., ethyl N-methyl-N-nitrosocarbamate) which do not contain a β -chloroethyl group attached to nitrogen. Second, interaction of KB-16 or the homologous ethyl ester with α -amino acids results in the disappearance of amino groups, and, in the case of cysteine, of the sulfhydryl group as well. Substances analogous to the "one-armed" sulfur and nitrogen mustards are presumed to be intermediates in these reactions, and conceivably may be toxic by virtue of the alkylating power of their β -chloroethyl groups. The β -chloroethyl group of KB-16 itself is relatively unreactive and neither of the above-described reactions corresponds to the principal mode of interaction of the sulfur and nitrogen mustards with amino, sulfhydryl, and other physiologically important groups (Chapter 19). The difference in mechanism is further emphasized by the fact that KB-16 reacts in nonaqueous media with the amino group of benzylamine, whereas reactions of the sulfur and nitrogen mustards depend upon a preliminary solvolytic activation in water.

The reaction of KB-16 with hemoglobin *in vitro* supplies a model for possible reactions of toxicological significance, and the absence of a comparably vigorous reaction with egg albumin suggests that the effects of the agent in the cell may be confined to only some of the biologically important molecules and reactive groups. Biochemical studies do, in fact, reveal that some enzyme systems are readily poisoned by KB-16, whereas others are not.

In one study with enzyme systems *in vitro*, the effects of KB-16 were compared with those of H.¹⁸ The three tested systems were inhibited by KB-16, but not so effectively as by H; previously hydrolyzed

KB-16 was without effect. Purified yeast hexokinase was inhibited 60 per cent by 0.006 *M* KB-16 and 50 per cent by 0.003 *M* H. Phosphocreatine phosphokinase was not significantly inhibited by 0.002 *M* and was inhibited 26 per cent by 0.006 *M* KB-16, whereas H at 0.001 *M* produced an inhibition of 90 per cent. Inorganic pyrophosphatase was inhibited 70 per cent by 0.001 *M* H and only 35 per cent by 0.002 *M* KB-16.

The respiration (oxygen consumption) of slices of tissue from a variety of organs was inhibited by treatment with 0.001 *M* KB-16.²⁵ In general the inhibition was greater (even complete) in the absence of added oxidizable substrates than in the presence of glucose, lactate, pyruvate, or other carbohydrate intermediates. The degree of inhibition increased with time in some instances. In contrast with its effect on oxygen consumption, KB-16 had but a slight effect on glycolysis as measured by carbon dioxide output or lactic acid production. Some but not all aspects of the metabolism of pyruvic acid by tissue slices were markedly affected by KB-16. Oxidation of pyruvate (utilization in presence of oxygen) was inhibited, but considerable species and organ variation occurred. The dismutation of pyruvate as measured by its utilization by chopped brain in the absence of oxygen was inhibited to a smaller extent, and its decarboxylation by dried yeast was unaffected. The synthesis of carbohydrate from pyruvate by kidney slices (rat) was almost completely inhibited by 0.001 *M* KB-16, but another condensation reaction, the synthesis of acetoacetate from pyruvate by chopped pigeon liver, was almost unaffected. Experiments with rat kidney indicated that the oxidative deamination of natural amino acids (i.e., glutamic) is greatly inhibited by KB-16 but that *D*-amino acid oxidase is unaffected. KB-16 had little effect on the oxidation of citrate and fatty acids by various preparations. Cholinesterase (Stedman) was inhibited by KB-16 but the agent had no significant effect on a number of other enzymes including the following: carboxylase, succinic dehydrogenase, cytochrome oxidase, choline oxidase, pepsin, and urease.

In summary, the primary effects of KB-16 seem to be due to the inactivation of certain essential proteins. Prominent among the sensitive substances appear to be the activating proteins of pyruvic oxidase and *L*-amino acid oxidase. Inasmuch as the reactions appear to be irreversible, the combatting of injury by KB-16 should be based primarily on prevention of the reactions.

TABLE 8. Properties of KB-16, mustard gas (H), and *tris*(β -chloroethyl)amine (HN3) bearing upon their utility as chemical warfare agents.

Property	KB-16	Agent H	HN3
Storage stability	poor	good	excellent
Explosion stability	questionable	good	good
Factors influencing stability on moist terrain:			
Solubility in water (ppm at room temperature)	7,000	500	80
Half life in water (min at 25 C)	?	8 $\pm$	2.4 $\pm$
Volatility (mg/l at 25 C)	0.87	0.96	0.12
Freezing point (C)	<— 50	14.3* 5–9†	— 3
Density (g/ml at 25 C)	1.21	1.27	1.24
Median detectable conc. (μ g/l)	7 $\pm$	0.6† 1.8*	15 $\pm$
Relative eye-injuring potency	1 $\pm$	1	1 $\pm$
Relative vesicant potency of liquid on not visibly sweating skin	<0.25	1	0.25–0.5
Relative vesicant potency of vapor on sweating skin	?	1	0.6–0.9

* Pure H.

† Levinstein H.

As is the case with H and the nitrogen mustards, instillation of very small amounts of KB-16 into the eye results in an inhibition of mitosis in the corneal epithelium.^{24b} This effect is exerted by less than one-thousandth of the minimal dose causing clinically visible lesions.

8.5 EVALUATION AS WAR GASES

KB-16 and the most toxic related compounds (i.e., ethyl N-(β -chloroethyl)-N-nitrosocarbamate and N-(β -chloroethyl)-N-nitrosoacetamide possess insufficient storage stability to be seriously considered for large-scale manufacture for purposes of chemical warfare. It has been suggested that this difficulty might be overcome by nitrosating the stable intermediate, methyl N-(β -chloroethyl)carbamate, with nitrous gases just before use, or by development of a munition designed to effect the nitrosation shortly before firing or even thereafter. However, comparison of the other properties of KB-16 with those of such persistent agents as H and HN3 (see Table 8) leads to the conclusion that KB-16 does not possess sufficient general utility to merit such special treatment. Moreover, in the opinion of the authors, it would not

deserve serious consideration even if a method for its stabilization should be forthcoming.

KB-16 does possess certain desirable features — low freezing point, lack of pronounced odor, and effectiveness as an eye-injuring agent at low dosages. The available data do not permit the conclusion that the vapor dosages necessary to produce casualties among unmasked troops by eye or respiratory injuries would be of a different order than the dosages required in the cases of H and HN3. Given equivalent low vapor dosages, however, KB-16 because of its less pronounced odor would be a more insidious and therefore more effective agent than H. On the other hand, it would not have this advantage over HN3, which is less odorous.

Because of the necessity of assuming that enemy troops will be equipped with gas masks, current doctrine gives greater weight to the vesicant effects than to the eye-injuring potency or inhalation toxicity of a persistent agent not having either much less odor or much greater potency (or both) than H, KB-16, or HN3. Thus, the relatively low vesicant potency of KB-16 places it at a great disadvantage in comparison with H.

Chapter 9

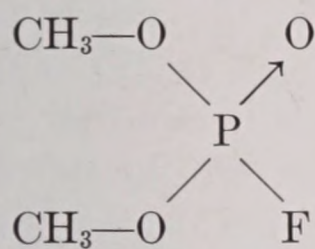
FLUOROPHOSPHATES AND OTHER PHOSPHORUS-CONTAINING COMPOUNDS

By Marshall Gates and Birdsey Renshaw

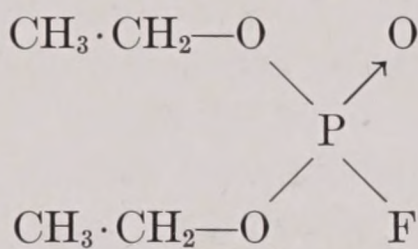
9.1 INTRODUCTION

APPROXIMATELY 200 phosphorus-containing compounds of widely varying structures were examined as candidate chemical warfare agents during World War II. Only the few represented by the dialkyl fluorophosphates, the diamidophosphoryl fluorides, the alkyl cyanamidophosphates, and the alkyl fluorophosphonates have merited detailed examination. The individual compounds that have received most attention are:

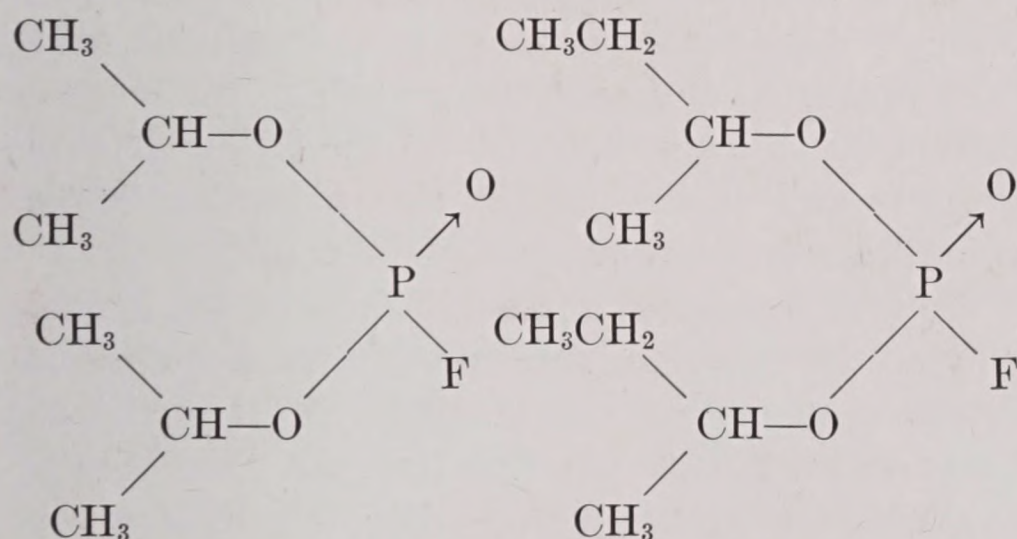
1. Dialkyl fluorophosphates.



Dimethyl fluorophosphate
(PF-1, TL 311, T-1035)

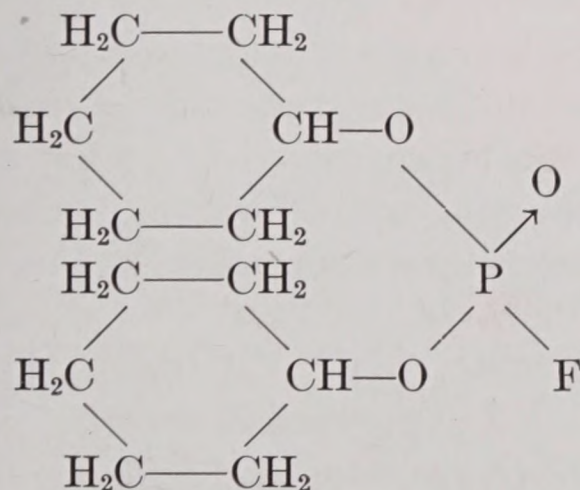


Diethyl fluorophosphate
(TL 345, T-1036)



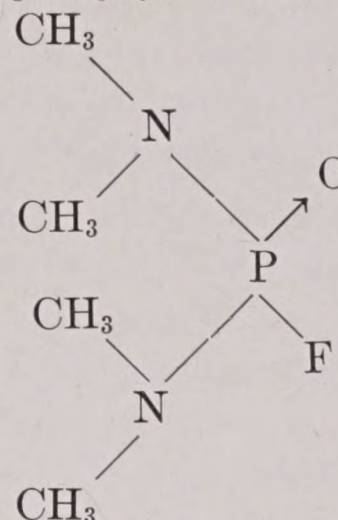
Diisopropyl fluorophosphate
(PF-3, DPF, TL 466, T-1703,
1152)

Di-sec-butyl fluorophosphate
(TL 1266, T-1835)



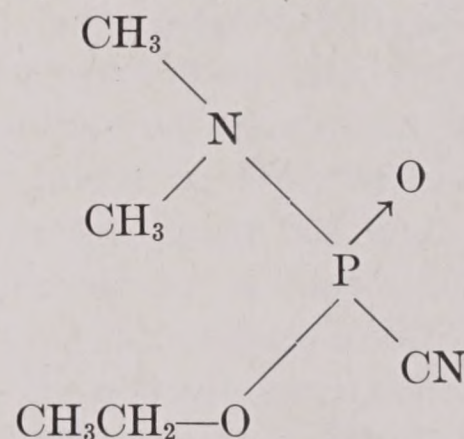
Dicyclohexyl fluorophosphate (TL 941, T-1840)

2. Diamidophosphoryl fluorides.



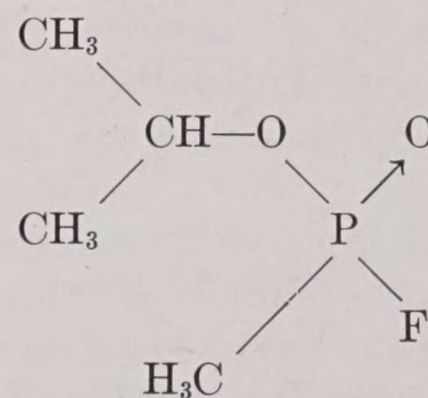
bis(Dimethylamido)phosphoryl fluoride (TL 792, T-2002)

3. Alkyl cyanamidophosphates.

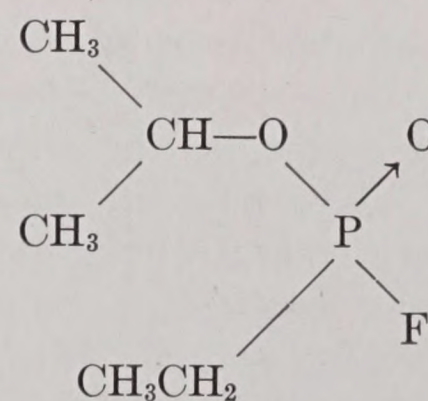


Ethyl dimethylamidocyanophosphate
(MCE, Tabun, Le-100, TL 1578, T-2104)

4. Alkyl fluorophosphonates.



Isopropyl methanefluorophosphonate
(MFI, Sarin, T-144, TL 1618, T-2106)



Isopropyl ethanefluorophosphonate
(TL 1620, T-2109)

The dialkyl fluorophosphates were described in the open literature in 1932. The British undertook their examination as war gases in 1941 and much work on them was subsequently carried out in the United Kingdom and United States.

They are parasympathetic stimulants and cholinesterase poisons of high potency. For some species (e.g., the monkey), PF-3 and di-*sec*-butyl fluorophosphate are more toxic than any of the standard United States or British chemical warfare agents. At lethal concentrations they are "quick-kill" agents, their action being only slightly less rapid than that of hydrogen cyanide (AC). However, their relatively low volatility, at 25 C 30 mg/l for PF-1, 8 mg/l for PF-3, and 1.8 mg/l for di-*sec*-butyl fluorophosphate, puts them in a class with the persistent agents and would render difficult the rapid administration of a lethal dose under field conditions. Chief interest in them has arisen from their action on the eye. They produce extreme constriction of the pupil, interference with the muscles of accommodation, potentially dangerous congestive iritis, and severe pain behind the eyes. PF-3 and di-*sec*-butyl fluorophosphate at a dosage of 50 mg min/m³ produce pupillary constriction, and PF-3 at about 300 mg min/m³ produces the other harassing symptoms just mentioned. However, by 1943 and 1944 careful assessments led to the conclusion that in practice these effects would be harassing rather than casualty producing. It is believed that troops supplied with gas masks would not become casualties from attack with the fluorophosphates except under circumstances where standard nonpersistent agents would have equally or more severe consequences. A useful interim summary of work on the fluorophosphates was prepared by Division 9 in 1944.³⁷

The diamidophosphoryl fluorides proved to be about as toxic as the fluorophosphates but to be less potent in their action on the eye. Their chief point of interest is that they are extremely stable in water and upon oral administration are among the most toxic of the known synthetic compounds.

The dialkyl fluorophosphates appear to be eclipsed in toxicological potency and potential value as chemical warfare agents by the alkyl cyanamidophosphates and alkyl fluorophosphonates. These compounds, known collectively as Trilons (a name assigned to them by the Germans), first came to the attention of United States and British workers after the termination of hostilities in Europe in the spring of 1945. It was then discovered that the Germans had

manufactured large quantities of MCE for use in bombs and high explosive-chemical shell. They had been attempting also to prepare MFI on a large scale but had been unable to overcome difficulties in its synthesis.

The Trilons are similar in mode of action to the fluorophosphates but are considerably more potent both in terms of inhalation toxicity and in the production of eye effects. For the monkey the $L(Ct)_{50}$'s of MCE and MFI are in the order of 250 and 150 mg min/m³, respectively. In man MCE at the extraordinarily low dosage of 3.2 mg min/m³ produces pupillary constriction. Dosages in the order of 15 to 20 proved to be definitely harassing because of ocular and systemic effects, and it would seem that 30 mg min/m³ might suffice to produce significant partial disability. Quantitative eye data on MFI are not available to the reviewers. Although MCE is somewhat less volatile than mustard gas (H) and is susceptible to hydrolysis, MFI has the rather high saturation concentration of 16 mg/l at 25 C and is quite stable. Moreover, it is virtually odorless.

It would seem that the Trilons are the one new group of chemical agents discovered during World War II which merit serious consideration for adoption as standard agents. Their use in high explosive-chemical shell, indistinguishable on detonation from ordinary high-explosive munitions, should be carefully evaluated and assessment made of the relative casualty-producing effects of (1) the initial cloud of droplets and vapor and (2) the subsequent vapor evolution from the contaminated terrain.

Division 9 has participated in work on the Trilons only to the extent of performing limited studies on synthesis, detection, and analysis. Most of the reports on work done by other agencies have become available during the period when the division was terminating its activities. Some of these reports may not have come to the attention of the reviewers. It has not been possible to render the review of the Trilons as complete as the discussion of the other agents of major importance. A summary of the field trials conducted at Raubkammer after the defeat of Germany has not been included, and a complete assessment of the value of the Trilons as chemical warfare agents has not been undertaken in this chapter.

9.2 SYNTHESIS AND PROPERTIES

9.2.1 Synthesis

Many methods have been used in the synthesis of the compounds listed in Table 1. The following dis-

TABLE 1. Fluorophosphates, amidocyanophosphates, fluorophosphonates, and other phosphorus compounds examined as candidate chemical warfare agents.

The compounds are arranged in the following general classes: (1) derivatives of phosphine, (2) derivatives of primary phosphines, (3) tertiary phosphines, (4) oxygen, sulfur, and nitrogen derivatives of trivalent phosphorus, (5) phosphorus pentahalides and related compounds, (6) phosphoric and phosphonic acid derivatives and their sulfur analogs, (7) quaternary phosphonium salts, and (8) miscellaneous compounds.

The following abbreviations are used: n_D^t , refractive index at t C; d^t , density in g/ml at t C; $d_{42}^{t_1}$, specific gravity at t_1 C in reference to water at t_2 C; mp, melting point in C; bp^p, boiling point in C at p mm Hg; vp^t, vapor pressure in mm Hg at t C; and vol^t, saturation concentration (volatility) in mg/l at t C.

Centigrade scale is used throughout the table.

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
1. Phosphorus trifluoride	10, 106c, 106d	bp	101.1°	106d	11, 106a
	...	mp	151.5°	106d	...
2. Phosphorus monochlorodifluoride	...	...		...	11
3. Phosphorus dichloromonofluoride	...	...		...	11
4. Phosphorus tricyanide	30d	bp ^{0.5} (sublimes)	150°	30d	...
5. Phenylphosphine	27g	...		...	11, 18
6. Ethyldichlorophosphine	2	bp ⁷⁶⁰	94–97°	2	11
7. Ethyldicyanophosphine	27r	bp ¹⁸	94–96°	27r	11, 69d
8. Phenyldichlorophosphine	...	...		...	...
9. Phenyldicyanophosphine	27d	d^{27}	1.1666	27d	11, 18
	...	bp ¹	100°	27d	...
	...	bp ²⁰	145°	27d	...
	...	mp	35°	27d	...
10. Phenyldithiocyanophosphine	27f	...		...	11, 18
11. <i>p</i> -Chlorophenyldichlorophosphine	...	...		...	...
12. <i>p</i> -Tolyldichlorophosphine	...	...		...	...
13. α -Naphthyldichlorophosphine	...	...		...	...
14. 2-Dibenzofuryldicyanophosphine	27h	...		...	11
15. 3-(N-Ethylcarbazole) dichlorophosphine	27h	...		...	11
16. 2-Phenoxthiindicyanophosphine	27h	...		...	11
17. Trichloromethylphosphine	27f, 77	...		...	11, 18
18. Triethylphosphine	27d	bp ⁷⁴⁴	127.5°	27d	11
19. Tributylphosphine	27b	...		...	...
20. Trioctylphosphine	27d	bp ⁵	234–237°	27d	11, 18
	...	mp	30°	27d	...
21. Tridecylphosphine	27h	...		...	11, 18
22. Diethylphenylphosphine	27f	...		...	11, 18
23. Diallylphenylphosphine	27f	...		...	...
24. Dibutylphenylphosphine	27e	d^{25}	0.9115	27e	11, 18
	...	bp ⁵⁰	185°	27e	...
	...	bp ^{0.5}	116°	27e	...
	...	fp	25°	27e	...
25. <i>tris</i> (2-Furyl)phosphine	29	...		...	11
26. <i>tris</i> (5- <i>tert</i> -Butyl-2-furyl)phosphine	...	bp ³	175°	34a	...
	...	mp	97–98°	34a	...
27. β -Chloroethoxydifluorophosphine	...	...		...	11
28. Phenoxydifluorophosphine	27n	...		...	11
29. β -Fluoroethoxydichlorophosphine	30f	...		...	11, 18
	...	bp ³⁰	50°	30f	...
	...	bp ⁷⁶⁰	140–145°	30f	...
30. β -Chloroethoxydichlorophosphine	27i	...		...	11, 18
31. β -Chloroethoxydicyanophosphine	...	...		...	11, 18
32. 2-Methyl-2-nitropropoxydichlorophosphine	...	...		...	11
33. 2-Methyl-2-nitropropoxydicyanophosphine	27r	...		...	11
	...	mp	35–45°	27r	...
34. (β -Chloroethylthio)dichlorophosphine	...	...		...	11, 18
35. (β -Chloroethylthio)dichlorophosphine	27r	n_D^{20}	1.5822	27r	11
	...	d^{30}	1.367	27r	...
	...	bp ^{0.8}	127–130°	27r	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
36. (β,β' -Dichloroisopropylthio)dichlorophosphine	27o	n_D^{20}	1.5286	27o	11
	...	d^{20}	1.5285	27o	...
	...	bp ¹	70–71°	27o	...
37. Dimethylaminodifluorophosphine	10	d^{25}	1.075	10	11
	...	bp	50°	10	...
38. Diethylaminodifluorophosphine	27n	...		...	11
39. Diethylaminodichlorophosphine	27d	d^{15}	1.196	27d	11
	...	bp ¹⁴	72–75°	27d	...
	...	...	189°/atmos.	27d	...
40. N,N-bis(β -Chloroethyl)aminodichlorophosphine	1, 27m	...		...	11
41. Ethyl-(β -chloroethylthio)chlorophosphine	27p	...		...	...
	...	bp ^{0.4}	89–92°	27p	11, 18
42. Diphenyl- β -chloroethylthiophosphine	27o	n_D^{29}	1.652	27o	11, 18
	...	d^{29}	1.248	27o	...
	...	bp ^{<1}	148–150°	27o	...
	...	bp ²	158–162°	27o	...
43. Diethoxyfluorophosphine	105q	bp ¹⁸	80–81.5°	105q	104r
44. <i>o</i> -Phenylenedioxyfluorophosphine	27h	...		...	11, 18
45. <i>bis</i> (β -Chloroethoxy)chlorophosphine	27i	...		...	11, 18
46. <i>o</i> -Phenylenedioxychlorophosphine	27g	...		...	11, 18
47. <i>bis</i> (β -Chloroethylthio)chlorophosphine	82	bp ¹	74–75°	82	82
48. <i>bis</i> (Dimethylamino)fluorophosphine	10	d^{24}	0.975	10	11
	...	bp	120°	10	...
49. Ethyl- <i>bis</i> (β -fluoroethoxy)phosphine	35a	bp ^{0.5}	40–49°	35a	11
50. Phenyl-diethoxyphosphine	27g	...		...	11, 18
51. Phenyl- <i>bis</i> (<i>o</i> -chlorophenoxy)phosphine	27h	...		...	11, 18
52. Ethyl- <i>bis</i> (β -chloroethylthio)phosphine	27p	n_D^{28}	1.5600	27p	11, 18
	...	bp	115–120°	27p	...
53. Phenyl- <i>bis</i> (methylthio)phosphine	27h	...		...	11, 18
54. Phenyl- <i>bis</i> (β -chloroethylthio)phosphine	27l	...		...	11, 18
55. <i>p</i> -Dimethylaminophenyl- <i>bis</i> (β -chloroethylthio)-phosphine monoethylate	27n	...		...	11, 18
56. Phenyl- <i>bis</i> (β,β' -dichloroisopropylthio)phosphine	27q	...		...	11
57. Phenyl- <i>bis</i> (butylthio)phosphine	27h	...		...	11, 18
58. Dimethyl hydrogen phosphite	27h	...		...	11, 18
59. <i>bis</i> (β -Fluoroethyl) hydrogen phosphite	...	bp ^{1.7}	109–110°	104p	104p
60. Diisopropyl hydrogen phosphite	...	bp ¹⁷	82.5°	104f	104f
61. Trimethoxyphosphine	27h	...		...	11, 18
62. Triethoxyphosphine	27e	d^{20}	0.968	27e	11, 18
	...	bp ⁷⁴⁰	155–156°	27e	...
	...	bp ¹⁴	36–38°	27e	...
63. <i>tris</i> (β -Fluoroethoxy)phosphine	30d	bp ^{0.5}	100–103°	30d	11
64. <i>tris</i> (β -Chloroethoxy)phosphine	27f	...		...	11, 18
65. <i>tris</i> (β -Bromoethoxy)phosphine	27m	...		...	11, 18
66. Tributoxyphosphine	34b	d^{25}	0.9257	34b	11
	...	bp ¹²	124–125°	34b	...
67. <i>tris</i> (β -Chloroethylthio)phosphine	27j, 82	bp ^{0.6}	82–83°	82	11, 18, 82
68. <i>tris</i> (β -Bromoethylthio)phosphine	27o	d^{30}	1.874	27o	11, 18
	...	bp ^{<1}	78–80°	27o	...
69. <i>tris</i> (Propylthio)phosphine	...	bp ¹⁵	173–176°	104f	104f
70. N,N- <i>bis</i> (β -Chloroethyl)amino- <i>bis</i> (β -chloroethylthio)phosphine	27m	...		...	...
71. <i>bis</i> (Diethylamino)fluoroethoxyphosphine	30f	bp ²⁵	108–111°	30f	11
72. <i>tris</i> (Piperidino)phosphine	27h	...		...	11, 18
73. Phosphorus pentafluoride	106b	bp	– 84.5°	108	106b, 106c
	...	mp	– 93.7°	108	...
74. Phosphorus pentachloride	...	...		...	92
75. Phenylphosphorus tetrafluoride	27f	...		...	11
76. Phenylphosphorus dibromodichloride	27h	...		...	11
77. Phosphoryl fluoride	106c	bp	– 39.8°	108	11, 104j
78. Phosphoryl chlorodifluoride	...	...		...	11
79. Phosphoryl bromodifluoride	...	...		...	11

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
80. Phosphoryl dichlorofluoride	105c, 105e	bp	52–57°	28c	11, 106c
81. Phosphoryl dibromofluoride	...	...		...	11
82. Phosphoryl chloride	...	...		...	92
83. Chlorocarbonylmethanephosphonylchloride	...	...		...	11
84. 2-Chlorohexene-1-phosphonyl chloride	27j	...		...	11
85. Diphenylphosphinyl chloride	27n	...		...	11, 18
86. Trimethylphosphine oxide	27i	...		...	11, 18
87. Triethylphosphine oxide	27d	bp	240°	27d	11, 18
	...	mp	51°	27d	...
88. Thiophosphoryl fluoride	106b	bp	– 52.3°	108	...
	...	mp	– 148.8°	108	...
89. Thiophosphoryl chlorodifluoride	...	...		...	11
90. Thiophosphoryl bromodifluoride	...	...		...	11
91. Thiophosphoryl dichlorofluoride	...	...		...	11
92. Thiophosphoryl dibromofluoride	...	...		...	11
93. Thiophosphoryl chloride	...	...		...	11
94. Thiophosphoryl bromide	...	...		...	11
95. Phenylthiophosphonyl chloride	27e	d^{13}	1.376	27e	11, 18
	...	bp ⁷⁴⁰	270°	27e	...
	...	bp ¹³	144°	27e	...
96. Triphenylphosphine sulfide	...	...		...	11
97. Trinaphthylphosphine sulfide	...	...		...	...
98. <i>tris</i> (<i>x</i> -Amylnaphthyl)phosphine sulfide	...	...		...	11
99. Triphenylphosphine selenide	...	...		...	11
100. Ethyl difluorophosphate	28e, 105f	d^{30}	1.25°	105f	11, 104g
	...	...	85–86°/atmos.	105f	...
101. Phenyl difluorophosphate	...	bp ⁸⁰	95°	104f	104f
102. Ethyl dichlorophosphate	105f	bp ¹⁵	71°	104g	104g
103. 2-Methyl-2-nitropropyl dichlorophosphate	...	...		...	11
104. N,N-Dimethylamidophosphoryl fluoride	10	d^0	1.2823	10	11
	...	bp ⁷⁶⁰	122°	10	...
105. N-Isopropylamidophosphoryl fluoride	27n	...		...	11
106. N,N-Diethylamidophosphoryl fluoride	27n	...		...	11
107. N,N- <i>bis</i> (β -Chloroethyl)amidophosphoryl fluoride	27m	...		...	11, 18
108. N-Ethylamidophosphoryl chloride	30b	bp ¹⁵	122–123°	30b	11
109. N- β -Chloroethylamidophosphoryl chloride	30b	bp ¹	146°	30b	11
110. N,N- β -Chloroethylmethylamidophosphoryl chloride	27j	...		...	11, 18
111. N,N-Diethylamidophosphoryl chloride	30b	bp ⁸	94.5–96°	30b	11
112. N,N- β -Chloroethylethylamidophosphoryl chloride	27k	...		...	11, 18
113. N,N- <i>bis</i> (β -Chloroethyl)amidophosphoryl chloride	30a	mp	54°	30a	11, 18
114. N,N-Dimethylamidocyanophosphoryl chloride	25	bp ^{0.01}	53–55°	25	69c
	...	n_D	1.4478	25	...
115. Ethyl difluorothiophosphate	28e	bp ⁷⁶⁰	78–79°	28e	11
116. Ethyl chlorofluorothiophosphate	...	...		...	11
117. Ethyl dichlorothiophosphate	28e	bp ²⁰	68°	104g	11, 18, 104g
118. N,N-Diethylamidothiophosphoryl chloride	27d	d	1.105	...	11, 18
	...	bp ²	83–86°	27d	...
	...	bp ¹⁵	100°	27d	...
119. Isopropyl ethanechlorophosphonate	35b	bp ¹	52°	35b	69d
120. Dimethyl fluorophosphate (PF–1)	See text	bp	145–148°	28a	See text
	...	vol ²⁰	22.9	12	...
121. Methyl ethyl fluorophosphate	17	bp ¹³	53.8–55.5°	17	11, 19
	...	n_D	1.3643	17	...
	...	vol ²⁵	20.7	69c	...
122. Diethyl fluorophosphate	28e, 105b, 105c, 105e	bp ⁷⁶⁰	169.8	28d	See text
	...	bp ²⁵	76–77°	105f	...
	...	vol ²⁰	8.18	12	...
123. β -Chloroethyl ethyl fluorophosphate	28i	bp ¹	70.5°	28i	11
	...	vol ²⁰	0.599	12	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
124. <i>bis</i> (β -Fluoroethyl) fluorophosphate	30e, 105j	bp ¹³	125–127°	105j	104m
	...	bp ^{0.5}	90–95°	30e	...
125. <i>bis</i> (β -Chloroethyl) fluorophosphate	28i, 105k	bp ¹⁵	142–144°	105k	11, 18, 104j
	...	bp ^{0.49}	88–89°	28i	...
126. <i>bis</i> (Ethylthio) fluorophosphate	105f	bp ¹⁵	104–107°	105f	104g
127. Diallyl fluorophosphate	...	...			104b, 105a
128. Dipropyl fluorophosphate	28h, 105e	bp ²⁰	98–100°	105e	104b
129. Diisopropyl fluorophosphate (PF-3)	See text	n_D^{25}	1.3780	105e	See text
	...	bp ²⁵	84–85°	105e	...
	...	bp ⁷⁶⁰	183° (est)	105e	...
	...	fp	– 93°	...	...
	...	vol ²⁰	5.84	12	...
130. <i>bis</i> (β, β' -Dichloroisopropyl) fluorophosphate	105k	bp ^{0.7}	163–165°	105k	...
131. Dibutyl fluorophosphate	28h	...		...	105a
132. Di- <i>sec</i> -butyl fluorophosphate	105f, 105l	bp ^{0.8}	62–64°	105f	See text
	...	vol ²⁰	1.12	12	...
133. Diamyl fluorophosphate	...	...		...	104b
134. Diisoamyl fluorophosphate	105f	bp ²⁰	145–148°	105f	104b
135. <i>bis</i> (α -Ethylpropyl) fluorophosphate	105k	bp ^{2.0}	97–98°	105k	104j
136. Dicyclohexyl fluorophosphate	105i	n_D	1.4558	28l	See text
	...	bp ^{0.3}	116°	105i	...
	...	vol ²⁰	0.0044	12	...
137. <i>bis</i> (1,3-Dimethylbutyl) fluorophosphate	105k	bp ^{2.7}	102–103°	105k	104j
138. <i>bis</i> (2-Methylcyclohexyl) fluorophosphate	105p	bp ^{0.1}	120°	105p	105p
	...	bp ^{0.15}	137°	105p	...
139. <i>bis</i> (α -Carbethoxyethyl) fluorophosphate	105k	bp ^{0.6}	126–128°	105k	104j
140. Diphenyl fluorophosphate	28j, 105f	bp ^{0.07}	106–108°	105f	104g
141. <i>bis</i> (Triethyllead) fluorophosphate	105e	mp	>200°	105e	105e
142. Diethyl chlorophosphate	...	...		...	11, 18, 104b
143. <i>bis</i> (β -Fluoroethyl) chlorophosphate	30d	bp ^{0.5}	108–112°	30d	...
144. Diethyl cyanophosphate	105r	bp ¹⁴	95–97°	105r	105r
145. Diethyl thiocyanophosphate	105e	bp ¹⁵	115–125°	105e	104d
146. Ethyl N-phenylamidofluorophosphate	105n	mp	50°	105n	105n
147. Methyl N,N-diethylamidochlorophosphate	25	n_D	1.4443	25	69d
	...	bp ^{0.13–0.14}	49–49.2°	25	...
148. Ethyl N,N-dimethylamidocyanophosphate (MCE)	21, 25, 105r	n_D	1.4243	25	See text
	...	d^{20}	1.077	25	...
	...	bp ^{0.3}	56–58°	25	...
	...	fp	50.0°	60	...
	...	vol ²⁵	0.567°	69c	...
	...	vol ²⁵	0.612	60	...
149. Methyl N,N-diethylamidocyanophosphate	35b	bp ^{0.5}	65–66°	35b	69d
150. Ethyl N,N-diethylamidocyanophosphate	30c	...		...	69c
151. Methyl N,N- <i>bis</i> (β -chloroethyl)amidocyanophosphate	...	...		...	69d
152. <i>bis</i> (Dimethylamido)phosphoryl fluoride	10, 105m, 105o	d^{25}	1.110°	10	See text
	...	bp ¹⁵	86°	105m	...
	...	bp ²	50°	10	...
	...	vol. ²⁰	2.16	12	...
153. <i>bis</i> (Butylamido)phosphoryl fluoride	105m	mp	59.5°	105m	104o
154. <i>bis</i> (Diethylamido)phosphoryl fluoride	105m	n_D^{16}	1.4321	28m	104o
	...	bp ^{2.5}	83–87°	28m	...
	...	bp ²⁰	124.5–125.5	105m	...
155. <i>bis</i> (Morpholido)phosphoryl fluoride	105q	mp	40°	105q	104o
156. <i>bis</i> (Piperidido)phosphoryl fluoride	105q	bp ^{0.3}	145°	105q	...
157. <i>bis</i> (Anilido)phosphoryl fluoride	105o	mp	143–144°	105o	104o
158. <i>bis</i> (Cyclohexylamido)phosphoryl fluoride	105m	mp	127°	105m	104o
159. <i>bis</i> (Benzylamido)phosphoryl fluoride	105m	mp	96°	105m	104o
160. <i>bis</i> (Methylanilido)phosphonyl fluoride	105m	bp ^{0.08}	163–165°	105m	104o
161. <i>bis</i> (Dicyclohexylamido)phosphoryl fluoride	...	...		...	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
162. <i>bis</i> (Dimethylamido)phosphoryl chloride	...	...		...	104r
163. Diethyl fluorothiophosphate	28g	bp ¹⁰	55°	28g	11, 18
164. Isopropyl methanefluorophosphonate (MFI)	See text	<i>n</i> _D ²⁵	1.3790	69d	See text
	...	<i>d</i> ^{25.6}	1.0941°	69d	...
	...	bp ¹⁵	56.5–57°	25	...
	...	vol ²⁵	16.4	69e	...
165. Isopropyl ethanefluorophosphonate	22	<i>n</i> _D ²¹	1.3872	22	See text
	...	<i>n</i> _D ²⁵	1.3817	69e	...
	...	<i>d</i> ²⁵	1.0552	69e	...
	...	bp ¹⁸	67–68°	22	...
	...	vol ²⁵	11.6	69e	...
166. 2-Chlorohexene-1-phosphonic acid	27g	...		...	...
167. Dimethyl methanephosphonate	27l	...		...	11, 18, 69a
168. <i>bis</i> (β-Chloroethyl) methanephosphonate	27l	...			11, 18, 69a
169. Di- <i>sec</i> -butyl fluoromethanephosphonate	105k	bp ³	96–100°	105k	104j
170. Diethyl 2-fluoroethanephosphonate	105s	bp ¹⁴	200–202°	105s	105s
171. Diethyl 2-chloroethanephosphonate	27k	...		...	11
172. Dimethyl propane-2-phosphonate	27l	...		...	11, 18, 69a
173. <i>bis</i> (β-Chloroethyl) propane-2-phosphonate	27l	...		...	11, 18, 69a
174. Diethyl α-toluenephosphonate	105s	bp ¹⁴	155°	105s	...
175. Diethyl carbethoxymethanephosphonate	27e	<i>d</i> ⁰	1.139	27e	11, 18
	...	bp	259°(atmos)	27e	...
	...	bp ¹²	149–150°	27e	...
176. β-Chloroethyl diethyl phosphate	...	bp ¹⁸	144–145°	104f	104f
177. <i>tris</i> (β-Chloroethyl) phosphate	...	...		...	77
178. <i>tris</i> (β,β'-Dichloroisopropyl) phosphate	...	...		...	77
179. <i>tris</i> (<i>o</i> -Cresyl) phosphate	...	...		...	77
180. <i>tris</i> (2-Methyl-6-propylphenyl) phosphate	...	...		...	77
181. <i>tris</i> (Ethylthio) phosphate	...	bp ¹⁶	172–174°	104f	104f
182. Diethyl amidophosphate	...	bp ^{0.2}	131–138°	104f	104f
	...	mp	45.5°	104f	...
183. Diethyl N-ethylamidophosphate	30b	bp ^{0.1}	96°	30b	...
184. Diethyl N,N-diethylamidophosphate	30b	bp ⁸	96°	30b	11, 18
185. Dimethyl N,N- <i>bis</i> (β-chloroethyl)amidophosphate	...	...		...	69d
186. Diethyl N,N- <i>bis</i> (β-chloroethyl)amidophosphate	30c	bp ¹⁰	164–165.5°	30c	11
187. <i>bis</i> (β-Chloroethylthio) N,N- <i>bis</i> (β-chloroethyl)amido-phosphate	27o	<i>n</i> _D ²⁵	1.5525	27o	11, 18
	...	<i>d</i> ²⁵	1.472°	27o	...
	...	bp ^{0.01}	155–160°	27o	...
188. <i>tris</i> (Dimethylamido)phosphate	10	bp ²	83°	10	11
189. Trimethyl thiophosphate	27h	...		...	11, 18
190. Triethyl thiophosphate	...	bp ²⁰	106°	104g	104g
191. Phosphonium iodide	27d	sublimes	62.5°	27d	...
192. <i>tetrakis</i> (Chloromethyl)phosphonium chloride	27e	mp	192–193°	27e	11, 18
193. β-Chloroethyltriethylphosphonium iodide	27g	...		...	11
194. β-Bromoethyltriethylphosphonium bromide	27c	mp	235°(d)	27c	11, 18
195. Triethylphenylphosphonium iodide	27h	...		...	11, 18
196. Triethyl- <i>p</i> -tolylphosphonium iodide	27h	...		...	11, 18
197. Triallylphenylphosphonium bromide	27g	...		...	11, 18
198. Triphenylphosphobetaine	27h	...		...	11, 18
199. β-Chloroethyltriphenylphosphonium iodide	27h	...		...	11
200. β-Bromoethyltriphenylphosphonium bromide	27d	mp	268°	27d	11, 18
201. 3-Chloroacetyltriphenylphosphonium chloride	27h	...		...	11, 18
202. β-Chloroethyl- <i>tris</i> (2-furyl)phosphonium iodide	27i	...		...	...
203. Ethyl metaphosphate	...	...			104c
204. Triphenylphosphorusphenylimine	27h	...		...	11, 18
205. Phosphonitrilic chloride	...	...		...	11

cussion will be limited to methods for the preparation of members of those series in which compounds of high toxicity are encountered and to methods for the

synthesis of a few closely related compounds. These include the fluorophosphates, fluorophosphonates, cyanophosphates, and amidophosphoryl fluorides.

DIALKYL FLUOROPHOSPHATES

Members of this series were first prepared by Lange¹¹⁷ by the action of alkyl iodides on silver fluorophosphate, which in turn had been prepared from ammonium fluorophosphate. The overall yield obtained was poor, approximately 12 per cent in the case of diethyl fluorophosphate,^a and the method is not suitable for preparations on a large scale. Lange observed the characteristic mitotic action of the dialkyl fluorophosphates. Detailed study of this class of compounds was initiated by the British during World War II. The class has now been thoroughly explored both by British teams and workers of the National Defense Research Committee [NDRC]. Most of the American synthetic work was carried out by NDRC Division 10.

A number of other methods have been used to prepare alkyl fluorophosphates. These methods include:

1. The action of alcohols or phenols on phosphoryl dichlorofluoride.

2. The action of phosphoryl chloride on alcohols to give dialkyl chlorophosphates, which are then fluorinated with sodium fluoride or hydrogen fluoride.

3. Chlorination of dialkyl hydrogen phosphites (rarely trialkyl phosphites) to dialkyl chlorophosphates, followed by fluorination as in the preceding method.

The first and third of these methods have found the widest application. That based on phosphoryl dichlorofluoride appears to be quite general but suffers from the disadvantage that the fluorination of phosphoryl chloride (by antimony trifluoride, calcium fluoride, or hydrogen fluoride) gives only poor yields of the desired dichlorofluoride.^{28k,105c,i,116} The method has been limited to laboratory preparations. The dialkyl hydrogen phosphite method is somewhat less general, but in cases where it is applicable excellent yields are obtained and it is well suited for scaling up to semitechnical production.⁴

The second method, based on the action of phosphoryl chloride on alcohols, has limited usefulness since it has not been possible to obtain satisfactory yields in the first step, i.e., in the preparation of dialkyl chlorophosphates.^{28c}

Direct esterification of fluorophosphoric acid to produce dialkyl fluorophosphates has not been successful.^{28b}

^a Improvements on Lange's preparation of ammonium fluorophosphate from ammonium bifluoride and phosphorus pentoxide have been reported by Marquina.¹¹⁸

Representative examples of preparations illustrating these methods are given below.

1. *Phosphoryl dichlorofluoride method — synthesis of dicyclohexyl fluorophosphate.* A mixture of phosphoryl chloride and antimony pentafluoride held at 75 C and 190–200 mm Hg is treated slowly with antimony trifluoride. The resulting volatile material is trapped and fractionated to give phosphoryl dichlorofluoride, b.p. 52–56 C, in a yield of 34 per cent. The procedure given is a modification of that of Booth and Dutton.¹¹⁶ Yields as high as 33 per cent based on phosphoryl chloride have been obtained using hydrogen fluoride as the fluorinating agent.^{28k}

A well-cooled solution of phosphoryl dichlorofluoride in dry ether is treated with cyclohexanol. After complete removal of hydrogen chloride, the resulting dicyclohexyl fluorophosphate is isolated in 50 per cent yield by fractional distillation under diminished pressure.¹⁰⁵ⁱ Attempts to prepare this compound by the phosphite or the phosphoryl chloride methods have been unsuccessful.^{28j}

The following compounds have been prepared by this method:

diethyl fluorophosphate^{105b}
dipropyl fluorophosphate^{105e}
diphenyl fluorophosphate^{105f}
bis(ethylthio) fluorophosphate^{105f}
bis(β -fluoroethyl) fluorophosphate^{105j}
bis(β -chloroethyl) fluorophosphate^{105k}
bis(β -methylcyclohexyl) fluorophosphate^{105P}
dicyclohexyl fluorophosphate^{28l,105i}

2. *Phosphoryl chloride method — synthesis of dimethyl fluorophosphate (PF-1).* Phosphoryl chloride is treated with methanol at –78 C and the mixture is allowed to come to room temperature slowly. Hydrogen chloride is evolved rapidly for a period of 2 hours. The mixture is transferred to a copper vessel and treated with hydrogen fluoride. Fractionation following a crude distillation gives PF-1 in 34.5 to 38.8 per cent yield.^{28c}

The following compounds have been prepared by this method:

dimethyl fluorophosphate^{28c}
diethyl fluorophosphate^{28c}
diisopropyl fluorophosphate^{28e}
bis(β -chloroethyl) fluorophosphate²⁸ⁱ

A slight modification of the method has been used to prepare ethyl β -chloroethyl fluorophosphate²⁸ⁱ and methyl ethyl fluorophosphate.¹⁷ By using thiophosphoryl chloride as a starting material, derivatives of fluorothiophosphoric acid have been prepared, and, by the use of one mole of alcohol per mole of phosphoryl or thiophosphoryl chloride in the first step, derivatives of difluorophosphoric or difluorothiophosphoric acid.^{28e,g,105f}

3. *Dialkyl hydrogen phosphite method — synthesis of diisopropyl fluorophosphate (PF-3).* A cooled solution of isopropyl alcohol in either carbon tetrachloride or ether is treated with a solution of phosphorus trichloride in the same solvent and then blown with air and treated with ammonia to remove hydrogen chloride. Filtration and fractionation give diisopropyl hydrogen phosphite in 82.5–89 per cent yield. This material is then chlorinated in 71–80 per cent yield to give diisopropyl

chlorophosphate, which is purified first by blowing to remove hydrogen chloride and then by fractional distillation. Fluorination is accomplished by gentle heating of diisopropyl chlorophosphate in benzene with powdered sodium fluoride. PF-3 is obtained in 84 per cent yield; the overall yield is thus in the order of 60 per cent. Hydrogen fluoride can also be used as a fluorinating agent.^{28f} Distillation of the intermediates can be eliminated and the whole process carried out in the original solvent (carbon tetrachloride) without greatly decreasing the yield.^{105h}

The following compounds have been prepared by this method:

dimethyl fluorophosphate ⁴
 diethyl fluorophosphate ^{105d}
 diisopropyl fluorophosphate ^{4,56,105g}
 di-*sec*-butyl fluorophosphate ^{105e}
 diisomyl fluorophosphate ^{105f}
bis(1,3-dimethylbutyl) fluorophosphate ^{105k}
bis(α -carbethoxyethyl) fluorophosphate ^{105k}
bis(α -ethylpropyl) fluorophosphate ^{105k}
bis(β,β' -dichloroisopropyl) fluorophosphate ^{105k}
bis(β -fluoroethyl) fluorophosphate ^{105j}

The last step of the reaction can be modified so as to replace chlorine by groups other than fluorine. Diethylthiocyanophosphate and diethyl amidophosphate have been prepared in this way.^{105e}

Semitechnical syntheses of PF-1 and PF-3 have been carried out by the dialkyl hydrogen phosphite method, but only with the latter compound have enough runs been made to standardize conditions. A description of the procedures used follows.

1. *Semitechnical preparation of diisopropyl fluorophosphate (PF-3).*⁴ The main reaction was carried out in a 130-gallon Lastiglas-lined jacketed vessel equipped with a lined and coated gas inlet pipe, a propeller-type stirrer, a charging pipe, sight glasses, manometer connections, and a bottom outlet. Steam or refrigerating brine could be circulated through the jacket. The reactor was connected to a 10-foot Lastiglas-lined steel tower, 6 inches in diameter, which was fitted with a lead coil condenser from which distillate could be passed to either of two receivers. The bottom outlet of the reactor was connected to the top of a 40-gallon filter tank equipped for vacuum filtration of the slurry and return of the filtrate either to the reactor or one of the receivers. Since plugs developed at the bottom outlet in many runs, an additional connection between the gas inlet and the top of the filter tank was provided to allow transfer of the slurry by this route. All vacuum lines led to a 35-gallon separator tank which was connected to a three-stage steam ejector. Drain lines leading to a 40-gallon lead decontaminating tank were provided. A separate still was provided for benzene distillation.

In a typical run, 212 lb (3.54 pound-moles plus 1 per cent excess) of isopropyl alcohol (<0.2 per cent water) was cooled with brine to -5°C in the jacketed reactor. Phosphorus trichloride (160 lb, 1.16 pound-moles) was added gradually with cooling and stirring over the course of 4 hours, during which the temperature was not allowed to exceed 12°C . The system was kept under slightly diminished pressure (about 700 mm).

The mixture was then stirred for $\frac{1}{2}$ hour before applying

the full vacuum of the steam jet. Chlorine was passed into the reaction mixture at a rate of 12 pounds per hour with continued cooling. The end of the reaction (10 hours) was indicated by a drop in temperature, even though the rate of flow of chlorine was increased. A total of 122 lb of chlorine (1.72 pound-moles, 48 per cent excess) was used.

To remove excess chlorine, hydrogen chloride, and isopropyl chloride, the stirred mixture was kept under vacuum for 2 hours, during which time the temperature was gradually raised to 20°C by passing steam into the jacket of the reactor. Ten gallons of benzene was then added and distilled off under reduced pressure at a maximum temperature of 30°C . The last traces of hydrogen chloride were removed by adding an additional 10 gallons of benzene and distilling under reduced pressure at reactor temperatures not exceeding 50°C .

After cooling to 20°C , 19 gallons of benzene recovered from a previous run was added. Dry sodium fluoride (95 per cent pure, 123.4 lb, 2.8 pound-moles, 142 per cent excess) was introduced into the reactor through an inlet line by means of a funnel. The stirred slurry was heated to reflux during 1 hour and held at reflux for 4 hours; it was then cooled and filtered. After washing the filter cake with three 5-gallon portions of benzene, the filtrate and washings were combined, collected in the cleaned reactor, and distilled under reduced pressure. The benzene forerun containing about 2 per cent of product was collected to be used in the following run. One hundred and fifty-eight pounds (74 per cent of theory based on phosphorus trichloride) of PF-3 was obtained. The entire run required 44 hours. An additional 20 hours was necessary to decontaminate and dry the system in preparation for the next run.

Preliminary design and round cost estimates for a full-scale plant to produce 500,000 lb per month of PF-3 by a batch process have been drawn up using data obtained during operation of this pilot plant. It is estimated that the capital cost of the complete plant would be \$700,000. Estimated manufacturing costs are \$0.37 per pound of product, \$2,222,000 per manufacturing year.⁵

Round cost estimates for a plant producing PF-3 by a continuous process have also been prepared.⁶ Although fewer experimental data are available (the estimates are based on laboratory scale work only),⁴ a smaller capital outlay and lower operating costs seem possible.

A total of 13 kg of PF-3 has been prepared at the British Research Establishment at Sutton Oak by a batch process resembling that just described.¹⁰²

2. *Pilot plant preparation of dimethyl fluorophosphate (PF-1).* Three pilot plant runs on a process similar to that already described for PF-3 have been carried out to produce a total of 35 lb of PF-1. This experience was not sufficient to allow standardization of conditions, but it was found that the temperatures required, which are somewhat lower than those in the PF-3 process, could be maintained without difficulty. Because of mechanical difficulties, yields approaching those obtained in the laboratory (72 per cent) were not realized in these three runs.⁴

DIAMIDOPHOSPHORYL FLUORIDES

A number of compounds in this series have been prepared by the application of the following more or less straightforward methods.

1. The action of amines on phosphoryl dichlorofluoride.

2. The controlled action of amines on phosphoryl chloride followed by fluorination.

3. The action of amines on phosphoryl fluoride.

The first of these methods appears to be general. It is carried out by adding a solution of phosphoryl dichlorofluoride in ether, benzene, or toluene to four moles of the amine in the same solvent. After filtration from the precipitated amine hydrochloride, the product is isolated by distillation or crystallization. The following compounds have been prepared in this way:

dianilidophosphoryl fluoride ^{105f}

bis(dimethylamido)phosphoryl fluoride ^{10,105m}

bis(diethylamido)phosphoryl fluoride ^{28g,105m}

bis(butylamido)phosphoryl fluoride ^{105m}

bis(cyclohexylamido)phosphoryl fluoride ^{105m}

bis(methylanilido)phosphoryl fluoride ^{105m}

bis(benzylamido)phosphoryl fluoride ^{105m}

A modification of this method involving prior treatment of phosphoryl dichlorofluoride with one mole of alcohol has yielded ethyl N-phenylamido-fluorophosphate. ^{105m}

The second method also appears to be general, and avoids the use of the difficultly available phosphoryl dichlorofluoride. The fluorination of bis(alkylamido)-phosphoryl chlorides proceeds somewhat less readily than that of dialkyl chlorophosphates. The method has been used successfully with bis(anilido)phosphoryl fluoride and with bis(dimethylamido)phosphoryl fluoride. ^{105o}

The third method suffers from the disadvantage that a large part of the fluorine is wasted. It has been used to prepare bis(dimethylamido)phosphoryl fluoride. ¹⁰

ALKYL CYANOAMIDOPHOSPHATES

Although vague but persistent rumors of a new German gas, Trilon, reached Allied hands from time to time during World War II through intelligence channels, no reliable information as to the nature of this gas or gases became available to the Allies until the spring of the German surrender, when German munitions charged with a new agent were captured. The agent was very quickly identified as ethyl dimethylamidocyanophosphate (MCE) and an intensive study of it covering all phases of interest to chemical warfare was started. About the same time an intelligence team interviewing members of the staff of the I. G. Werke, Elberfeld, reported that this

compound had been discovered in 1937 by I. G. Elberfeld during a search for new insecticides, and that in the following year an even more toxic and insidious substance, isopropyl methane-fluorophosphate, had been discovered. ⁷² Both compounds had been reported to the War Ministry under its standing order to the German chemical industry regarding the reporting of toxic substances.

The laboratory method of synthesis of ethyl dimethylamidocyanophosphate (MCE), disclosed in detail by the I. G. representatives, made use of the following steps. ⁷²

1. The interaction of two moles of dimethylamine and one of phosphorus oxychloride, first at 30 C and finally at 120 C, to produce dimethylamidophosphoryl chloride in 95 per cent yield.

2. The action of sodium cyanide and ethanol on dimethylamidophosphoryl chloride to give MCE in 90 per cent yield.

This procedure has been checked in at least two laboratories in this country ^{24,25} and the German claims substantially confirmed, although the yields obtained were not so high. No detailed study of the reactions was carried out. A novel alternative method for laboratory preparation of the agent has been used in Great Britain. ¹⁰⁵ In this procedure, diethoxyphosphorus chloride is allowed to react with dimethylamine and the resulting diethoxydimethylaminophosphorus is treated with cyanogen iodide to give MCE directly.

In 1939-40 the Germans began pilot plant production of MCE at Munsterlager, near Bremen, and experienced no difficulty in the manufacture of 50 tons of the material. Construction of a large plant at Dyhernfurth near Breslau was begun in January 1940, but production did not begin until April 1942. ^{72,73} In the plant process, chlorobenzene was used as a reaction medium in the final step. Initially the product was stripped to a content of approximately 5 per cent of chlorobenzene. Later a product containing 20 per cent chlorobenzene was standardized. Both 105-mm shells and 250-kg bombs were charged with the agent. ⁷³ A total of 10,000 to 12,000 tons ^{73,113} of MCE was produced. It is worth noting that the figure 12,000 tons represents 18 per cent of the total German production of war gases of all kinds, ¹¹³ which gives some indication of how largely this agent figured in the plans of the Germans.

MCE is a high-boiling, fairly stable liquid possessing a faint fruity odor. The pure material is colorless, but as technically produced MCE is dark brown.

It boils at 83 C under 1.5-mm pressure, at 120 C under 10-mm pressure, and at 230 C with some decomposition at atmospheric pressure.¹¹² Its density at 20 C is 1.077 and its refractive index (n_D^{20}) is 1.4240.⁶⁰ Its vapor pressure appears to be about one-half that of H₂¹¹² and can be represented as a function of temperature by the following equation:⁹⁰

$$\log_{10} P(\text{mm}) = 11.345 - \frac{3,750}{T}.$$

Its volatility at 25 C is 0.567 mg/l.^{69c} The tactical use of the agent as an aerosol produced by heavy-walled shell equipped with large bursting charges appears to have been envisaged by the Germans.

MCE is claimed by the Germans to be the optimum compound of this series as regards toxicological properties,⁷³ but this assertion has not been verified in this country since no comprehensive synthetic program was established to explore the field.

A related compound of relatively high toxicity was encountered during an attempt to prepare the isopropyl analog of MCE by the simultaneous action of sodium cyanide and isopropyl alcohol on dimethylamidophosphoryl chloride. In this case the cyano group alone was introduced, and dimethylamidocyanophosphoryl chloride was obtained in 68 per cent yield. Its toxicity is approximately one-half that of MCE.²⁵

ALKYL FLUOROPHOSPHONATES

Mention has been made of the discovery of this class in 1938 by members of the staff of I. G. Elberfeld. The optimum compound of the series, isopropyl methanefluorophosphonate (MFI), is several times as toxic for most species as is MCE, is more volatile, and is also more difficult to detect by odor. It aroused great interest among the Germans, but in spite of intensive efforts to develop manufacturing methods, production on a plant scale was never realized.

As first reported to intelligence teams, the laboratory preparation of MFI proceeded as follows.⁷²

Dimethyl hydrogen phosphite is prepared in 90 per cent yield by the action of methanol on phosphorus trichloride, and is converted into dimethyl methanephosphonate in 85 per cent yield by the action of metallic sodium followed by methyl chloride. Finally, methanephosphoryl chloride, produced by the action of phosphorus pentachloride on dimethyl methanephosphonate, is converted to MFI by the simultaneous action of sodium fluoride and isopropyl alcohol. The yields in these steps are 90 and 82 per cent respectively.

Attempts by both American and British groups to use this scheme without modification were not entirely successful. In this country yields greater than 14 per cent were not obtained in the methylation step even when methyl iodide was substituted for methyl chloride or when the reaction was carried out in an autoclave at 125 C. By using dimethyl sulfate, however, yields of 77 per cent were obtained in this step.²⁵ British workers were able to carry out the methylation step in 59 per cent yield by using a modification of the original German procedure in which dimethylhydrogen phosphite was alkylated by treatment with sodium sand in dry ether followed by methyl chloride.^{105s} Neither group obtained greater than 42 per cent in the final fluorination and esterification.

After this work was well under way, additional information became available from intelligence sources to the effect that the Germans had used sodium methoxide in methanol instead of metallic sodium in the methylation step^{72,73} and that two alternative methods for the final fluorination, one using sodium fluoride and the other hydrogen fluoride, were possible. The use of hydrogen fluoride made possible operation at lower temperatures but introduced corrosion problems. Few details on the actual operation of the final step are available.⁷³

The substitution of higher alcohols for methanol in the first step of this process appears to be advantageous. Dimethyl hydrogen phosphite is rather unstable, is water-soluble, and its sodium salt is insoluble in organic solvents. Diethyl hydrogen phosphite has given better results in the hands of British workers, particularly in the methylation step,^{25,105s} whereas the use of butanol to give dibutyl hydrogen phosphite followed by methylation with dimethyl sulfate and sodium methoxide was adopted as optimum for a simplified process suitable for pilot plant use by NDRC workers.²⁴ In the latter example, the solubility of sodium dibutyl phosphite in organic solvents appears to be distinctly advantageous. By this method methanephosphoryl chloride can be obtained in 79 per cent overall yield.²⁴ Other improvements made during this study were substitution of a water-wash for filtration to remove sulfate salts after the methylation, and combination of the first three steps to eliminate all distillations except that of methanephosphonyl chloride.

The isomerization process of Arbusow¹¹⁵ has also been used to prepare dialkyl methanephosphonates. Dimethyl methanephosphonate is obtained in 95 per cent yield by heating trimethyl phosphite with

methyl iodide,^{105s} whereas a similar reaction using tributyl phosphite yields 89 per cent of dibutyl methanephosphonate.²⁴

A novel process well suited for conversion to plant scale operations has been developed on a laboratory scale for the synthesis of the ethyl analog of MFI. In this process tetraethyllead is allowed to react under nitrogen with phosphorus trichloride to give 89 to 96 per cent of the theoretical yield of ethylphosphorus dichloride, which is then converted in 85 to 95 per cent yield to ethanephosphoryl chloride by the action of sulfuryl chloride. Treatment with sodium fluoride and isopropyl alcohol converts this substance into isopropyl ethanefluorophosphonate in 72 to 85 per cent yield. The first two steps can be carried out in the same vessel.²² The resulting ethyl analog of MFI has about three-fourths the toxicity of MFI itself. No attempt has been made to synthesize MFI by a similar process using tetramethyllead, which is reputed to be much less easily handled than tetraethyllead.

Pilot plant production of MFI has not been undertaken in this country. The efforts of the Germans to produce this substance on a plant scale were not successful. Although intermediates for the material were made in substantial quantity (300 tons of dimethyl hydrogen phosphite, 5 to 10 tons of dimethyl methanephosphonate, and 1 to 2 tons of methanephosphoryl chloride were produced), not more than $\frac{1}{2}$ ton of MFI itself was produced.^{73,113} Corrosion appeared to have been the principal source of difficulty. Equipment shortages necessitated the use of resin-coated equipment where stainless-steel or glass-lined equipment would ordinarily have been used. Silver-lined equipment was resorted to in some cases.^{72,73}

MFI is a colorless, almost odorless liquid boiling at 59 C at 8 mm of mercury. Its volatility at 25 C is 16.4 mg/l.^{69e} It is less stable than MCE, but can be stabilized by the addition of 0.5 per cent of diethylamine.

9.2.2 Chemical Reactions, Detection, and Analysis

Studies on the chemistry, detection, and analysis of phosphorus compounds as candidate chemical warfare agents have been limited almost exclusively to PF-3, certain of its close relatives, and MCE.

DIALKYL FLUOROPHOSPHATES

Solutions of PF-1 in 0.9 per cent saline lose virtually all toxicity in 3 hours. This deterioration is re-

tarded by buffering the solutions near neutrality but is markedly accelerated by buffering at pH 9.7.^{26g}

PF-3 is hydrolyzed slowly at room temperature by water to give fluoride ion and diisopropyl phosphoric acid. This hydrolysis is less than 50 per cent complete in 15 hours and is still incomplete after 23 hours.³ In neutral aqueous solutions at body temperature the half-hydrolysis time is about 9 hours.⁸⁶ In 2 per cent aqueous alkali PF-3 is rapidly hydrolyzed at room temperatures, although more concentrated alkalis appear to retard this hydrolysis.^{105e} *bis*(Dimethylamido)phosphoryl fluoride appears to be considerably more stable to hydrolysis than PF-3.⁸⁶

In contrast to the ease with which fluoride ion is freed by aqueous alkalis, the isopropyl groups of PF-3 are very resistant to alkaline hydrolysis. For example, no isopropyl alcohol can be detected after refluxing with 10 per cent sodium hydroxide for 72 hours.^{31a} Advantage is taken of this resistance to hydrolysis in several of the analytical procedures for PF-3 based on determination of fluoride ion, the titration of which is interfered with by phosphate ion but not by alkyl phosphates.^{7,8,105t}

The kinetics of hydrolysis of PF-3 have been studied in several laboratories.^{16,31b,85} In addition to the marked catalysis by alkali already noted, the reaction is also acid-catalyzed, and thus in pure water is autocatalytic. In buffered solutions the hydrolysis is pseudomonomolecular. The observation of a pronounced acceleration by phosphate ion suggests that the decomposition may be subject to general base catalysis as well as acid catalysis, although acetate ion is the only other anion which has been observed to have an accelerating effect.²⁰

When hydrolysis of PF-3 is allowed to proceed in acid solutions, the course of the reaction may become complex. For example, in some experiments, acetone and isopropylphosphorous acid were formed in addition to fluoride ion, and no phosphate ion could be detected. Other dibasic acids were likewise absent. Acetone is also formed when acid solutions of diisopropylphosphoric acid are treated with sodium fluoride. It has not always been possible to reproduce these experiments, however, and the mechanism by which acetone and isopropylphosphorous acid are formed is not yet clearly understood.²⁰

PF-3 does not react with sodium hypoiodite to give iodoform and does not react with thiosulfate ion.^{31b,c}

Methods for the detection and analysis of compounds of the fluorophosphates series are summarized in Chapters 34 and 37. The following general

remarks may be supplemented by reference to these chapters.

The fluorine atom of PF-3 and related compounds is readily converted to fluoride ion on hydrolysis and any detection methods depending upon the recognition of fluorine ion are thus applicable to these compounds. The ability of fluoride ion to bleach metallic lakes of certain dyes or its etching effect on glass has been utilized for recognition.^{14,41,42,58,94,95,106e}

A device making use of the etching effect has been examined by the British.^{95,96,97}

The decomposition of volatile fluorine compounds by hot platinum filaments or hot platinized silica gel to produce hydrogen fluoride is applicable to members of the fluorophosphate series.^{14,58}

Detection of PF-3 collected upon plain silica gel tubes can be accomplished by testing either for fluoride ion or for phosphate ion after suitable treatment. The DB-3 reagent may also be used.¹³

Chemical methods for the detection of fluorine compounds, including PF-3, in water have been developed.^{41,42} Use of the miosis produced by PF-3 as a method for detection of this agent in water has also been proposed. It is claimed that 25 to 50 ppm can be detected in 3 minutes by this method without injury to the eye.⁴⁰

The analysis of PF-3 has been accomplished by volumetric, colorimetric, or gravimetric determination of the fluoride ion produced by alkaline hydrolysis. Alternately, phosphate ion can be determined colorimetrically after vigorous acid hydrolysis with hydrobromic, hydriodic, or sulfuric acids.^{7,8,50,80,81,101,105e,t,106f}

Methods suitable for use in field and chamber analyses of PF-3 have been described.^{9,50,81}

ETHYL DIMETHYLAMIDOCYANOPHOSPHATE (MCE)

MCE is readily destroyed in either acidic or basic solutions.^{61,112} In alkaline solutions, cyanide ion is liberated rapidly even in the cold, the half life at 25 C being 5 minutes at pH 8.5 and 30 minutes at pH 7.5.⁶¹ In acid solutions rapid liberation of dimethylamine occurs, the half life in solutions of pH 1 being 2 minutes, that in solutions of pH 3, 90 minutes. The substance has maximum stability at pH 4.5, where its half life is 7 hours with respect to both cyanide ion formation and dimethylamine liberation. Solutions of maximum stability result from hydrolysis in unbuffered solutions, since the hydrolysis products are acidic and self-buffering in the range pH 4 to 5.⁶¹

In solutions of high acidity (i.e., 3 normal), hydro-

gen cyanide as well as dimethylamine is liberated rapidly but complete degradation to phosphoric acid results only from boiling the substance with mineral acids.¹¹²

Bleach and chlorinating agents react readily with MCE to yield CK.^{70,112}

MCE is extremely hygroscopic, and moist solutions of it slowly liberate AC.^{61,66h} Its faint fruity odor cannot be relied on for detection.^{91,114} Its median detectable concentration as determined with the osmoscope is 2.2 $\mu\text{g}/\text{l}$.^{66h}

The standard liquid vesicant detectors, both British and American, give positive reactions with MCE. This is true of the H papers of the kit, food testing, and of the M-6 paper, M-7 crayon, and M-5 detector paint of the United States Chemical Warfare Service, and of the British Detector, Gas, Ground. The British differential detector powder gives a yellow color with the agent.^{66g,70,112} The black dot (AC) tube of the M-9 detector kit has about the same sensitivity for MCE vapor as it has for AC itself (20 μg) but is considerably less sensitive than the German AC tube (sensitivity 2–3 μg). The red dot (nitrogen mustard) tube gives a nonspecific test.⁷⁰ The British pocket vapor detector gives no reaction with the agent.¹¹² The ready production of cyanide ion and a volatile amine on alkaline and acid hydrolysis, respectively, together with the production of phosphate ion on ultimate hydrolysis, can be taken as confirmatory identification.¹¹²

For field or chamber analysis, MCE can be collected in 1.25 normal sodium hydroxide and titrated with silver nitrate,^{23,62,66g} or (for small amounts) estimated colorimetrically with sodium picrate.^{23,66k,66l} Phosphorus colorimetry using molybdivanadophosphate is also suitable if the sample, collected in alkali, is fumed with perchloric acid or otherwise completely decomposed. The sensitivity of this method is several times as great as that of those already described.^{23,62} Attempts to adapt the DB-3 method to the analysis of MCE have not been entirely successful.^{66k}

9.2.3

Stability

DIALKYL FLUOROPHOSPHATES

PF-3 is stable when stored in glass at 25 C. When stored in steel at 65 C, slight decomposition takes place as indicated by sludge formation. This decomposition continues at an increased rate when the sample is removed and stored in glass at 25 C. This

effect may be due to the action of light and dissolved iron salts.⁵⁶

In the presence of steel at 58–60 C, diethyl fluorophosphate appears to stable for several months.^{28g}

Both PF-1 and PF-3 are resistant to flashing. No temperature has been found at which dimethyl fluorophosphate flashes; PF-3 can be made to flash feebly over a narrow temperature range.^{26o}

ETHYL DIMETHYLAMIDOCYANOPHOSPHATE (MCE)

Technical MCE containing 20 per cent monochlorobenzene is reported by the Germans to be stable even on prolonged storage.⁷³

It is also claimed by them that MFI, when stabilized with <1 per cent of diethylamine, can be stored in iron and that it is stable in methanol solution. It was supposed to have been used in such solutions.⁷³

9.2.4 Decontamination

DIALKYL FLUOROPHOSPHATES

Bleach suspensions and dry bleach react vigorously with PF-3 and presumably with other fluorophosphates, and normal field decontamination procedures as used for vesicants should be effective. The chloramides S-461 and S-328 do not react with PF-3 or diethyl fluorophosphate, nor do dilute solutions of calcium hypochlorite.^{3,20,94}

The ease of hydrolysis of the fluorine atom of the dialkyl fluorophosphates by water alone varies considerably with structure. PF-1 is 72 per cent hydrolyzed after standing 1 hour in water at 24 C; the diethyl compound, 24 per cent; and the PF-3, 1 per cent. However, dilute alkalies at room temperature produce rapid hydrolysis of all three esters.³ Lime slurry should thus be an effective decontaminant. Dilute solutions (approximately 0.4 per cent) of sodium hydroxide have been proposed for skin decontamination.

Mere hosing of contaminated areas with water should mitigate the vapor hazard produced by PF-3, since it is soluble to the extent of 1.5 per cent in water.⁹⁴

ETHYL DIMETHYLAMIDOCYANOPHOSPHATE (MCE)

In the Dyhernfurth plant of the Germans, equipment used for the synthesis of MCE was decontaminated by steam and ammonia. Surface decontamination, in the absence of steam, was done by solutions of ammonia or of amines.⁷²

Alkalies or bleach and water have been recommended by the Chemical Warfare Service for decontamination, but it is recognized that the production

of CK by the action of bleach on MCE might prove hazardous under some conditions.⁷⁰

9.2.5

Protection

DIALKYL FLUOROPHOSPHATES

Adequate protection against dialkyl fluorophosphates appears to be provided by United States, British, German, and Japanese canisters, and it is doubtful whether canister penetration by these agents will ever be a significant problem. Representative United States, German, and Japanese canisters have been tested against PF-1, PF-3, and methyl ethyl fluorophosphate; all afforded good protection.^{57,59} The standard United States Navy canister provides complete protection against PF-3 as does the British Lt. Mk. II canister.^{75,111}

ETHYL DIMETHYLAMIDOCYANOPHOSPHATE (MCE)

Completely adequate protection against the vapor of MCE is afforded by American, British, and German canisters^{62,70,72,90,112} and it is implied in intelligence reports that the German canister gives adequate protection against MFI.⁷² American canisters (M-11 and M-10A-1) give adequate protection against ethyl dimethylamidocyanophosphate as an aerosol (particle size 2 μ , concentration 100 μ g/l, flow rate 32 lpm), but the Canadian canister, which has a resin wool pad-type filter, allows serious penetration after 5 minutes.⁷⁰ It is to be noted that the tactical use of the agent contemplated by the Germans was as an aerosol.

Combined activated carbon-aeration treatment of water contaminated with MCE gives excellent removal of cyanide ion, odor, and color but does not remove organic phosphorus if the water has been standing more than 15 hours after contamination.^{66j,k}

9.3

TOXICOLOGY

9.3.1 Detectability by Odor and Other Physiological Signs

The Trilons and fluorophosphates may be detected by (1) odor, (2) a feeling of tightness in the chest and/or throat, and (3) pupillary constriction.

MCE and PF-3 have faint, sweetish odors. The available osmoscopic data for these and other agents are presented in Table 2. It is apparent that the fluorophosphates are relatively odorless. Crude MCE (German shell filling) is more readily detected but does not possess so pronounced an odor as H. MFI is said to be odorless, or practically so.^{104t}

TABLE 2. Detectability by odor of MCE, fluorophosphates, and other representative agents as determined by the osmoscopic technique.

Agent	Median detectable conc. ($\mu\text{g/l}$)	Reference
MCE (German shell filling)	2.2	66h
PF-3	36	49
Dimethyl fluorophosphate	18	49
Diethyl fluorophosphate	15	49
H (plant run Levinstein)	0.6	51
H (pure thiodiglycol)	1.8	65
HN3 (plant run)	15	66m
AC	34	39
CG	4.4	38

In man-chamber experiments a German shell filling containing MCE with 20 per cent monochlorobenzene was detected at a concentration of $1.6 \mu\text{g/l}$ by 2 of 10 subjects.⁸⁸ The pure agent seemed to be more odorous and was detected at a concentration of $0.35 \mu\text{g/l}$ by each of 4 subjects.⁸⁸ It is possible that the Germans considered decreased detectability by odor to be one advantage of the addition of monochlorobenzene to MCE. In one of the man-chamber experiments with PF-3, concentrations of 37 to $70 \mu\text{g/l}$ remained undetected by odor.⁵² In a field (annulus) test PF-3 could be detected at an average concentration of $0.5 \mu\text{g/l}$ but the odor was not sufficiently characteristic to be easily identifiable.

Man-chamber experiments indicate that throat irritation and a feeling of tightness in the chest are apparently more sensitive indicators of exposure to MCE and PF-3 than are the odors. In the case of MCE, 6 of 10 observers exposed to the German shell filling at a concentration of $1.6 \mu\text{g/l}$ experienced the feeling of chest constriction, as did each of the 4 who were exposed to $0.35 \mu\text{g/l}$ of pure MCE.⁸⁸ In the case of PF-3 each of 18 subjects exposed to $8.2 \mu\text{g/l}$ — not detected by odor — experienced throat irritation and a feeling of chest constriction within 60 to 90 seconds.⁷⁹

Pupillary constriction to pin-point size develops within a matter of minutes upon exposure to moderate dosages of MCE and PF-3, although it is longer delayed at the minimal effective concentrations (see the following paragraph).

The foregoing suggests that troops having masks available could protect themselves against dangerous dosages of MCE and PF-3 if they could take note of odor, feeling of chest constriction, and pupillary size. High concentrations could be detected quickly by odor or chest and throat signs, and the

mask donned before a large dosage had reached the eyes or lungs. Sufficiently low concentrations to escape these means of detection would be revealed after some minutes or an hour by pupillary constriction, and the mask applied if more prolonged exposure were unavoidable. Thus, except upon very sudden exposure to high concentrations of vapor and aerosol, dosages sufficient to produce systemic effects would seem to be theoretically avoidable. It is much more difficult to detect exposures to small dosages sufficient to produce miosis and the other harassing but not disabling symptoms described in Section 9.3.2, and it is in this sense that MCE and PF-3 may be considered insidious. Accidental exposures to undetected dosages that resulted in these symptoms are reviewed in the next section. It has been emphasized that PF-3 is readily absorbed by lacquer, rubber, clothing, and hair. The gradual desorption of vapor can result in obtaining, within confined spaces, concentrations which suffice to produce eye effects but which may remain undetected until these effects appear.^{37,52,100}

The lack of odor of MFI may not prove to be so great an advantage as would appear at first sight if throat irritation and feeling of chest constriction should prove to be definite indications of the inhalation of very low concentrations.

9.3.2

Eye Effects

The vapors of the fluorophosphates and Trilons are absorbed directly by the eye and produce contraction of the pupil (miosis) and interference with the muscles of accommodation. As a consequence harassment due to poor dim light vision in dim light and to pain and difficulty of focusing is experienced. A potentially dangerous congestive iritis can develop and pain behind the eyeball frequently becomes very severe. These ocular symptoms can be relieved by (repeated) instillations of a mydriatic (e.g., atropine) but the subject is left with a dilated pupil and paralyzed accommodation. The concomitant systemic effects often include a feeling of tightness in the chest, nausea, and vomiting. No data are available concerning the exposure of human subjects to dosages sufficiently large to produce more severe disability.

STUDIES ON ANIMALS

Tests of the effects of dialkyl fluorophosphates on the eyes of animals originally served (1) to demonstrate beyond reasonable doubt that cautious trials with human volunteers (see the next section) could

be carried out without risk of causing permanent eye damage, and (2) to determine the relative miotic potencies of some of the compounds.

Various observations have demonstrated that pupillary constriction is produced in rabbits and monkeys at dosages considerably smaller than those required to cause permanent ocular injury or marked systemic effects.^{26r,32a,33a,49,79,104a,b} The factor of safety in the case of PF-3 is most strikingly illustrated by experiments with rabbits. Instillation into the conjunctival sac of a nearly lethal dose of the liquid (i.e., 1.15 mg/kg) and repeated instillations of smaller doses, while eliciting intense miosis, lacrimation, and a transient increase of intraocular pressure, caused no permanent ocular injury.^{32a} Similarly, although vapor dosages of less than 1,000 mg min/m³ sufficed to induce marked pupillary constriction, dosages of 15,000 mg min/m³ caused no permanent damage.^{32a,104b} In the case of both PF-1 and PF-3 the vapor dosages necessary to produce miosis in the rabbit are considerably smaller than those required to kill.^{104c} With the monkey, a species that is exceptionally sensitive to the lethal actions of PF-3 and di-*sec*-butyl fluorophosphate, the difference between dosages producing miosis and serious systemic poisoning may be smaller,^{26m,p,q,r,63,64} as may also be the case with man (see next section).

Tests with rabbits have demonstrated that PF-3 is a markedly more potent pupillary constrictor than are the dimethyl, diethyl, dipropyl, or diallyl esters.^{49,104b} It not only produces constriction at lower dosages, but also for longer times.^{49,79,104b} Illustrative data are presented in Table 3.

TABLE 3. Relative miotic effects of several dialkyl fluorophosphates in rabbits.^{104b}

The animals were exposed for 3 minutes to nominal concentrations of 1/50,000 (0.11 to 0.16 mg/l).

Dialkyl ester of fluorophosphoric acid	Average per cent of initial pupil diameter		
	After 10 min	After 100 min	After 300 min
Dimethyl (PF-1)	32	82	100
Diethyl	27	58	85
Dipropyl	45	68	96
Diisopropyl (PF-3)	16	31	52
Diallyl	27	46	67

That di-*sec*-butyl fluorophosphate is a very potent miotic is revealed by the production of marked pupillary constriction within 10 minutes after the exposure of monkeys to 50 mg min/m³ ($t = 2$ minutes).^{26r} Animal data adequate to provide a basis for evalu-

ating the relative potencies of this compound and of PF-3 are not available. Dicyclohexyl fluorophosphate also appears to be an effective miotic that produces pupillary constriction after a somewhat greater latency than characterizes the compounds just mentioned.¹⁰⁴ⁱ Its potency relative to that of PF-3 is not known.

The high miotic potency of MCE is illustrated by observations on animals^{69c,88} but quantitative comparisons with the fluorophosphates are not available. At high doses MCE can produce conjunctival hemorrhages.⁸⁸

OBSERVATIONS ON HUMAN SUBJECTS

MCE appears to be considerably more potent in producing eye effects than any of the fluorophosphates.⁸⁸ Although a dosage of 0.7 mg min/m³ ($t = 2$ minutes) was without effect on the eyes, 3.2 mg min/m³ ($t = 2$ minutes) produced slight but definite miosis. Dosages of 14 to 21 mg min/m³ produced a severe harassing effect of several days' duration. The action of these dosages was characterized by the following symptoms, not all of which were observed in all the subjects: pin-point constriction of the pupils, lasting for several days; severe frontal headache; retrobulbar pain, tightness in the chest, and coughing; pain on focusing on near objects; slight blurring of both distant and near objects; slight blurring of peripheral visual fields; nausea and vomiting; engorgement of the bulbar conjunctival, anterior ciliary, and radial iris vessels, and of the vessels at the base of the iris; acute ciliary tenderness; and fall in intraocular tension. This symptomatology was usually almost completely relieved within an hour after the instillation of either atropine or hyoscine solution, but the effects of the treatment did not persist. In the absence of treatment the symptoms became most harassing 24 to 48 hours after exposure and persisted in gradually decreasing intensity for several days thereafter. In the case of one observer exposed to 30 mg min/m³ ($t = 10$ minutes), the harassment was very severe and, in addition to the effects mentioned above, visual acuity was markedly reduced and had not returned to normal 17 days after exposure.

Data on the eye effects of MFI are not available.

PF-3 produces symptoms similar to those caused by MCE but is definitely less potent. From the data presented below it would appear that exposure to 40 mg min/m³ of PF-3 vapor produces about the same effects as exposure to 3 mg min/m³ of MCE

vapor. At larger dosages, 200–400 mg min/m³ of PF-3 may correspond roughly to 14 to 20 mg min/m³ of MCE.

PF-1 and diethyl fluorophosphate are definitely less potent miotics than PF-3. *bis*(Dimethylamido)-phosphoryl fluoride is also less potent, probably much less so. Di-*sec*-butyl fluorophosphate appears to be somewhat more potent than PF-3 but definitely less potent than MCE.

The observations on which the above statements are based may be abstracted as follows.

1. *Ethyl dimethylamidocyanophosphate (MCE)*. The results of one controlled laboratory study are available.⁸⁸ In addition there have been accidents which demonstrate that exposures to undetected concentrations of the vapor can produce extreme pupillary contraction and in addition congestion of the eyes.^{66j, 71} In other instances a feeling of tightness in the chest has accompanied and given warning of the exposure.

Four subjects exposed in a man-chamber to 0.7 mg min/m³ ($t = 2$ minutes) detected the odor of the agent and experienced a brief feeling of tightness in the chest. They developed no miosis.

Ten additional subjects were exposed to a dosage of 3.2 mg min/m³ ($t = 2$ minutes). Only two noticed any smell. Six experienced a very slight feeling of constriction in the chest. Slight miosis developed in all after 30 to 60 minutes.

Ten subjects, some of whom had been exposed 4 hours previously in the preceding group, were exposed to 14 mg min/m³ ($t = 2$ minutes). The gas was detected faintly by smell and those not previously exposed felt a slight tightness in the chest. Soon after exposure all subjects had contraction of the pupils which persisted for 48 hours. Severe headache and pain in the eyes followed unless atropine was administered. Vascular injection of the eyeballs was present. Difficulties of focusing were experienced. Vomiting on the day after exposure occurred in four of the subjects.

Three additional subjects were exposed to 14 mg min/m³ ($t = 10$ minutes). The odor and a feeling of tightness in the chest were detected. Pupillary constriction, headache, rhinorrhea, nasal congestion, and other symptoms developed rapidly and persisted for several days in the absence of treatment. Visual acuity at moderate illuminations was not markedly affected.

Five additional subjects were exposed to 21 mg min/m³ ($t = 10$ minutes). They became severely harassed by the symptoms that developed. The symptoms and their times of onset (minutes, in parenthesis) were tightness in the chest (1.5 to 8), coughing (1.5 to 6), pin-point pupils (10), lacrimation (2 to 10), retrobulbar pain (8 to 19), conjunctival congestion (2 to 10), "tingling" of the eyelids (6 to 10), rhinorrhea (6 to 120), frontal headache (13 to 18), difficulty of seeing distant objects (11, 14 — two cases), difficulty in seeing near objects (15 — one case), and constriction of the peripheral visual fields (15 — one case).

One subject with one eye protected was exposed to 30 mg min/m³ ($t = 10$ minutes). The protected eye was unaffected. The pupil of the exposed eye began to contract within 4 minutes and had become fully contracted within 12 minutes.

Visual acuity in dim light had markedly deteriorated within an hour and had not fully recovered 17 days later. Moderate conjunctival and severe ciliary congestion had developed within 3 hours. The subject was unable to sleep for two nights because of severe pain above and behind the exposed eye.

2. *Dimethyl fluorophosphate (PF-1)*. At low dosages PF-1 is not so potent a harassing agent as PF-3, nor does the pupillary constriction which it induces persist as long. Although this ester is considerably less readily detected by odor than PF-3, it is more irritating to the throat and chest. In subjects exposed to nominal concentrations as low as 5.7 μ g/l (1/10⁶) it produced a tightening sensation in the throat.^{104a} No eye effects were noted when subjects were exposed, presumably for short times, to this concentration or to one four times as great.

At a considerably higher concentration, 114 μ g/l (1/50000), the throat sensation was not more marked but eye effects were produced: an exposure of 30 seconds' duration ($Ct = 57$ mg min/m³) produced in five of seven subjects some pupillary constriction and discomfort but no spasm of the muscles of accommodation; exposures of 1 to 5 minutes' duration ($Ct = 114$ to 570) produced within 5 to 10 minutes pupillary constriction lasting for an hour or more, and, in 50 per cent of the subjects, a marked spasm of the muscles of accommodation.

3. *Diethyl fluorophosphate*.⁷⁹ This ester also appears to be considerably less potent than PF-3. In twelve subjects 2-minute exposure to a nominal concentration of 139 μ g/l ($Ct = 278$ mg min/m³) produced throat irritation within 10 to 30 seconds, then a painless tightening sensation in the chest, and finally coughing toward the end of the exposure. Within 30 to 60 minutes the pupils had partially contracted and their reflexes to light and accommodation were absent. There was no significant alteration in visual acuity in daylight or in simulated twilight, although the sensitive Rangefinder Test⁷⁸ revealed harassment. The size and reflexes of the pupil had returned to normal within 18 hours. At no time was there more than minimal congestion of the iris in any of the subjects.

4. *Diisopropyl fluorophosphate (PF-3)*.^{32c, 36, 48, 49, 52, 67b, c, 76, 79, 84, 100, 104e}

a. *First (preliminary) British examination*.^{104e} Ten minutes after exposure of two subjects to a nominal dosage of 246 mg min/m³ (0.082 mg/l for 3 minutes), the pupils began to constrict and subsequently were reduced to pin-point size, with the result that the laboratory appeared dim. The observers experienced difficulty and pain in focusing, eye ache, and headache. A book could be read only if held within a few inches of the eye. The miosis and difficulty of accommodation persisted for 2 to 3 days in the case of the older volunteer (over 60 years of age) and for almost a week in the younger (28 years). The report does not mention extraocular symptoms.

Upon exposure of two additional subjects to a nominal dosage of 82 mg min/m³ (0.0082 mg/l for 10 minutes) the effects did not develop for about 30 minutes but then appeared as described above and persisted for 3 days. The subjects could read only with pain and difficulty. Vision in dim light was poor. Distant vision was impaired but recovered sometime before near vision had returned to nor-

mal. The eyes of one observer were congested for about a day beginning 1 day after exposure.

- b. *Preliminary American observations.*^{49, 52} Upon exposure of only the eyes of several subjects to a nominal dosage of approximately 300 mg min/m³ (0.1 mg/l for 3 minutes) only one subject reported slight subjective eye irritation during exposure. Miosis became maximal within 20 to 30 minutes and persisted for about a week. The subjects experienced difficulty in accommodation and found reading painful during the first 2 days; they had less difficulty after 1 to 2 days in spite of the maintenance of pupillary contraction and the development of irritation (congestion), eye ache, and headache. There was only a slight decrease in far vision; Snellen charts could be read about as well as before exposure. The subjects reported that their night vision was poor. Atropine and adrenaline instillations gave relief but had to be repeated daily. In two men accidentally exposed for several hours to low and undetected concentrations, a viewed object first appeared clearly but then rapidly became blurred, accommodation was slightly reduced, and nearsightedness apparently increased.⁴⁹

Four men whose eyes only were exposed to approximate dosages of 111 to 210 mg min/m³ (0.037–0.070 mg/l for 3 minutes) detected no odor and experienced no discomfort during exposure. They subsequently developed miosis, difficulty of focusing and blurred vision, eye ache and headache, conjunctivitis and a gritty sensation in the eye, and twitching of the eyelids. Visual acuity as tested by Snellen charts was not reduced. Four men accidentally exposed to low, undetected concentrations experienced eye effects as just described and, in two cases, nausea. There is some evidence that in two cases visual acuity in dim light was reduced.⁵²

- c. *Second British examination.*⁷⁹ Subjects were exposed to nominal concentrations of vapor in a large man-chamber and subsequently examined for pupil size, pupillary reflexes to light and accommodation, and acuity of near and distant vision as tested with Jaeger and Snellen Test Type indices both in daylight and in simulated twilight (approximately 0.4 footcandle).^b The general condition of their eyes was also examined and their performance on the Rangefinder Test^c in ordinary daylight and simulated twilight^b determined.

All six subjects exposed to 41 mg min/m³ (0.0082 mg/l for 5 minutes) complained of throat irritation about 1 minute after the start of the exposure and of "tightness in the chest" after about 1.5 minutes. Three hours later the pupil was only slightly contracted and pupillary reflexes to light and accommodation were present and normal. The tests for visual acuity revealed no significant deterioration either in ordinary light or in simulated twilight. The average degree of harassment for the group as measured by the Rangefinder technique was 36 per cent in ordinary light and 50 per cent in simulated twilight. At no time was there

^b This level of illumination was far greater than would be encountered at night and the tests therefore give no adequate measure of the handicaps which the subjects would have experienced in night fighting.

^c This technique has been described⁷⁸ and critically discussed.¹¹⁰

congestion of the iris and no subject experienced headache or other discomfort.

Upon exposure to 99 mg min/m³ (0.033 mg/l for 3 minutes) all of 18 subjects experienced throat irritation after about 50 seconds of exposure and complained of "tightening of the chest" within about 1.5 minutes. It took 4 to 6 hours for maximal miosis to develop, at which time pupillary reflexes were absent. The tests for visual acuity (as described) revealed no significant change although the Rangefinder test indicated 14 per cent harassment with ordinary lighting and 100 per cent in simulated twilight. Congestive iritis with accompanying headache and photophobia developed within 18 to 24 hours. Atropine sulfate (1 per cent) proved more effective than homatropine (1 per cent) in dilating the pupils and relieving the iritis.

Of 12 subjects exposed to 328 mg min/m³ (0.164 mg/l for 2 minutes) all experienced throat irritation and a feeling of tightness in the chest within 30 to 105 seconds. There was also some coughing, but no eye irritation, lacrimation, or blepharospasm occurred. The pupils were only partially contracted 30 minutes after exposure but had contracted nearly to pin-point size within 3 hours. Pupillary reflexes were then absent. A definite deterioration in acuity for distant vision had developed within 30 to 60 minutes after exposure and was not notably more marked when tested in simulated twilight (see preceding paragraph) than when tested at higher levels of illumination. The individual alteration in near vision was variable at both tested levels of illumination but for the group as a whole there was definite deterioration. Twenty-four hours after exposure distant vision had improved but near vision had deteriorated further. The subjects without exception complained of headache, the pain being referred to above or behind the eyes and being sufficiently intense to interfere with sleep. There was well-marked congestive iritis and conjunctival congestion but no edema of the lids, conjunctiva, or cornea. The average degree of harassment for the group as measured by the Rangefinder technique was 63 per cent in ordinary light and 100 per cent in simulated twilight.

Granted that the symptoms caused some discomfort and, at night, visual harassment, they were not considered to be of a disabling nature in the recorded opinion of the British Ophthalmic Panel and Medical Subcommittee.¹¹⁰

- d. *Third British examination.*⁸⁴ The eyes only of sixteen subjects were exposed for 5 minutes in a constant-flow device to analytically determined dosages of 40 to 250 mg min/m³. Subsequent clinical examination included observations on pupil size, visual acuity at high and relatively dim illuminations, the near point of accommodation, the threshold of scotopic vision, and the condition of the conjunctiva, cornea, and iris.

In summary, with dosages up to 191 mg min/m³ the effects produced by the vapor on pupil size and the accommodative mechanism were not considered of serious consequence. However the three subjects exposed to 250 mg min/m³ developed a congestive iritis associated with painful symptoms and consequently were considered to be partially disabled for 3 to 6 days.

The pupil had contracted to a minimum diameter of 1 to 2 mm within less than 1 day at all dosages and within 1 hour at dosages above 116 mg min/m³. The miosis began

to abate after 2 days at the lower dosages and after 3 days at the higher ones, but was not completely relieved for 5 to 6 days. During the first days prolonged dark adaptation resulted in no pupillary dilatation.

Visual acuity tested with Snellen charts in bright light (17 footcandles) showed practically no deterioration. Indeed, the uncorrected vision of myopes was improved, as a result of the small pupil size. Visual acuity in relatively dim light was determined by lowering the illumination of the Snellen chart until the smallest type which the subject could read at high illumination was no longer legible. Before exposure the average illumination recorded for the twelve eyes of six observers was 2.8 footcandles (range 0.32–10.5). Twenty-four hours after exposure to 40 or 80 mg min/m³ it was 6.4 footcandles (range 0.32–17). This change was not considered consistent or marked. It must be emphasized that the tested range of illuminations was sufficiently high that cone (not rod) vision was being measured, and that the results throw no light on the impairment which may have been produced in night vision.

Among subjects exposed to 40 to 191 mg min/m³ the near point of clear vision was brought in, indicating increased ciliary tension. However the absence of serious impairment of distant vision indicates that any existing spasm of the muscles of accommodation was not a serious handicap at the light intensities employed in the tests.

From the changes in pupil size caused by the PF-3 vapor one might have expected as much as a 16-fold rise in scotopic visual threshold. Actually the change in threshold brightness level, as measured with the Craik Adaptometer 1 to 2 hours after exposure to 116–191 mg min/m³, was only 2- to 10-fold (average 5+ fold in six subjects).

Subjects exposed to 100–191 mg min/m³ generally developed conjunctival hyperemia 2 to 3 days after exposure. At 250 mg min/m³ the hyperemia was much more severe and developed within 24 hours. In addition a marked and potentially dangerous congestive iritis, accompanied by painful symptoms, made its appearance.

Examination with the slit lamp revealed no corneal changes, nor were changes noted upon ophthalmoscopic examination in instances where miosis had been abolished with a mydriatic.

Among the subjective symptoms reported by the subjects were mistiness before the eyes, eye ache, and difficulty of seeing in the dark.

Instillation of homatropine (0.43 minim) had to be repeated three times at hourly intervals in order to obtain significant pupillary dilatation in two observers who developed congestive iritis following exposure to 250 mg min/m³. After the third instillation the congestive symptoms were relieved but paralysis of accommodation occurred and the observers became partially disabled because of blurry vision.

e. *American assessment.*^{32c,48,67b,c} One subject was exposed in a man-chamber to a dosage of 181 mg min/m³ ($t = 6.7$ minutes), eight subjects to 165 mg min/m³ ($t = 8.7$ minutes), one subject to 290 mg min/m³ ($t = 10.7$ minutes), and six subjects to 244 mg min/m³ ($t = 9$ minutes).

All the men exposed to 165 mg min/m³ experienced a slight feeling of tightness and constriction in the chest, apparent $\frac{1}{2}$ hour after exposure and particularly noticeable

several hours later. Instances of rhinorrhea, diarrhea, nausea, and vomiting (one case) occurred but, except for the rhinorrhea, may not have been due to the effects of the PF-3. No muscle tremors — a sign which might be expected to herald serious systemic poisoning — were observed.

Of the men exposed to 244 mg min/m³ (0.027 mg/l for 9 minutes), five of six experienced a fleeting feeling of chest constriction while in the chamber. This returned and persisted for 2 days, being accompanied by coughing in two cases. All the subjects developed rhinorrhea within an hour after the exposure. Only one developed nausea, and he vomited twice. There were no abdominal cramps or muscle tremors. One volunteer exposed to 290 mg min/m³ developed constant nausea for a day following exposure, experienced abdominal cramps, and exhibited increased nasal secretion. He had no muscular tremors and felt no chest constriction.

The majority of men in both groups had diminished distant vision, which was caused by a spasm of the muscles of accommodation. Although the resultant false myopia measured between 1.75 and 6.5 diopters, because of the small pupil size the visual acuity was not diminished greatly. The greater part of the diminution of distant vision had developed within 45 minutes after exposure. Further deterioration occurred at 3 hours in some cases. Recovery occurred at 2 to 7 days, being slightly more rapid in the subjects exposed to the smaller dosage than in those exposed to the larger.

Maximal miosis developed within 10 to 15 minutes after exposure. Among the men exposed to 165 mg min/m³, the pupils began to relax after 1 to 3 days and attained normal size and activity after 3 to 9 days. Among those exposed to 244 mg min/m³ relaxation did not begin until after the third day and complete recovery required 5 to 11 days.

All the volunteers showed a diffuse conjunctival injection which required 5 days to clear up.

Concurrently with the development of pupillary constriction and spasm of accommodation, the near point of accommodation moved to within 3 to 6 cm from the cornea, and it became increasingly difficult for the men to focus after gazing into infinity. Small type could be read but several seconds were required before it could be seen clearly. *Without exception the men complained of intense pain when they attempted to perform visual tasks within 18 inches.* Recovery of the untreated eyes gradually occurred over an average of 3.5 days after exposure to the lower dosage and 4.5 days after the larger.

Except for one man who exhibited a transient rise in intraocular tension, all displayed a subnormal tension for several days.

A performance test (in daylight) showed no decrease in efficiency of marksmanship and all of the men felt that they could competently discharge such military tasks as guard duty, vehicle driving, and rifle firing.

The reports state that prolonged questioning failed to elicit any symptoms of defective night vision, all the volunteers feeling that their visual acuity at night was proportional to that in the day. This statement is at variance with the results obtained in other tests and observations. It would not be anticipated that the vision of men with maximally

TABLE 4. Summary of toxicities.

Compound	$L(Ct)_{50}$ in mg min/m ³ (10 min nominal)			Increase of $L(Ct)_{50}$ with increase of exposure time	LD_{50} (mg/kg)	
	Mouse	Monkey	Range for all tested species		Rabbit, intra- venous	Mouse, percu- taneous
Ethyl dimethylamidocyanophosphate (MCE)	380	250	200-1,000	Slight	0.1 ±	1 ±
Isopropyl methanefluorophosphonate (MFI)	250	150	100-300	Definite	0.02	1 ±
Isopropyl ethanefluorophosphonate	330	200	150-700 ±	Definite	...	1.7
Dimethyl fluorophosphate (PF-1)	2,600	...	2,500- >12,000	Marked	3	36
Diethyl fluorophosphate	8,200	...	7,000- >14,000	?	...	35
Diisopropyl fluorophosphate (PF-3)	5,900	600	600- >8,000	Slight	0.4	72
Di-sec-butyl fluorophosphate	5,200	250 ± 150	250- >18,000	?	...	...
Dicyclohexyl fluorophosphate	1,100	...	1,000-8,000	?	...	...
bis(Dimethylamido)phosphoryl fluoride	950	...	950- >4,000	?	3 ±	...

contracted pupils would be normal at illuminations sufficiently low to confine visual function to the rods.

The serum cholinesterase concentration of all the subjects was reduced by the exposure to PF-3 to 1 to 5 per cent of the normal value.

- f. *Additional accidental exposures.*^{36,76,100} Workers accidentally exposed to undetected concentrations at the American pilot plant developed extreme miosis of 1 week's duration. Difficulty of night vision was stressed.³⁶

In a report on a similar incident at the British pilot plant emphasis was placed on pupillary contraction, blurring of vision, especially in artificial light, headache, and tightness in the chest.¹⁰⁰ In another incident⁷⁶ undetected exposures to vapor produced miosis, poor vision in dim light, difficulty in focusing, twitching of the eyelids, nasal discharge, and (in some cases) conjunctivitis. There was no mention of headache or chest symptoms.

5. *Di-sec-butyl fluorophosphate.*¹⁰⁵¹ This ester has been given only preliminary tests with four human subjects. Upon exposure to a nominal concentration of 1/10⁶ (Ct = approximately 45 mg min/m³) all noticed a tightness across the chest but three of the four felt that it was not sufficient to call for a respirator. About 5 minutes after the subjects left the chamber, miosis set in, became intense, and persisted for 5 days. Comparison with the results obtained with PF-3 at comparable and somewhat greater dosages^{79,104c} would indicate that the di-sec-butyl ester may be the more potent miotic agent.

6. *bis(Dimethylamido)phosphoryl fluoride.*^{104o} Exposure of four volunteer subjects to about 45 mg min/m³ (7.3 µg/l or 1/10⁶, for 5 to 7 minutes) produced no observable ocular or systemic effects. This compound is therefore less effective, perhaps very much less effective, than PF-3.

9.3.3

Toxicity

INHALATION AND INJECTION TOXICITIES

Toxicity data for the more intensively studied Trilons, fluorophosphates, and dialkylamidophosphoryl fluorides are given in Tables 5 through 17 and are summarized in Table 4. It is apparent that the Trilons are the most toxic volatile agents considered in

TABLE 5. Toxicity of ethyl dimethylamidocyanophosphate (MCE) by inhalation.

The animals were totally exposed to the vapor of the agent. Concentrations were nominal except when otherwise designated.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³)	Number of animals	Reference
Mouse	2	400	100	69d
	5	385	100	69d
	5	500-750	22	104s
	10	380	460	69d,e
	10	220*	140	87
	10	500-750	18	104s
	20	420	100	69d
	30	420	100	69d
	60	670*	120	87
	120	840*	60	87
Rat	5	750-1,000	14	104s
	10	750-1,000	10	104s
	10	500-1,500	14	69c,e
	10	304*	230	87
	20	385*	54	66k
	60	620*	60	87
	120	1,200*	120	87
	5	1,000 ±	6	104s
Guinea pig	10	1,000-2,000	6	104s
	10	393*	81	87
	10	500-1,500	12	69c,e
	60	740*	56	87
	120	1,500*	48	87
	10	1,000*	15	87
	10	>4,000	6	104s
	10	>2,000	4	69c,e
Rabbit	10-62	840*	55	66h
	7.5	300-800	3	104s
	10	250	8	69c,e
	10	400	4	69c,e
	10	700*	9	87
	14-23	400-700*	10	66h
	13-129	765*	30	66k
	20	1,400*†	38	66k
Monkey	5-10	400 ±	5	104s
	10	250	4	69c,e
	10	180*	3	87

* Analytically determined concentration

† Angora goats.

TABLE 6. LD_{50} 's of ethyl dimethylamidocyanophosphate (MCE).

The figures in parentheses are the number of animals used.

Route of administration	Species	Approximate LD_{50} (mg/kg)		Reference	
Intravenous	Mouse	0.15	(15)	69c	
	Rat	0.066	(35)	66h	
	Rabbit	0.0625	(46)	66h	
		0.18	(14)	87	
		0.125	(15)	104s	
	Dog	0.084	(20)	66i	
		0.146	(9)	69c	
Subcutaneous	Mouse	0.4	(25)	87	
	Rat	0.3–0.4	(42)	87	
	Guinea pig	0.2	(20)	87	
	Rabbit	0.5	(30)	87	
Percutaneous	Mouse	1.0	(70)	69c	
		>4	(20)	66g	
	Rat	18–35	(47)	87	
	Guinea pig	35	(43)	87	
	Rabbit	2.5–3.0	(5)	69g	
		3.3	(60)	69h	
		35	(19)	87	
	Dog	30–50	(4)	69c	
	Goat	>5	(2)	66g	
		1.1	(21)	66h	
	<i>Per os</i>	Monkey	3	(17)	87
			9.3	(6)	69c
		Rat	3.7	(107)	66h
8			(26)	87	
Rabbit		16.3	(51)	66h	
Dog		5–11	(12)	66h	

TABLE 7. Toxicity of isopropyl methanefluorophosphate (MFI) by inhalation.

The animals were totally exposed to the vapor of the agent. All concentrations were nominal.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³)	Number of animals	Reference
Mouse	5	230	100	69e
	10	250	100	69e
	10	150-250	22	104t
	15	345	120	69e
	20	360	100	69e
	30	420	100	69e
Rat	10	300	18	69d,e
	10	150-250	12	104t
Guinea pig	10	180	18	69d,e
	10	150-250	12	104t
Rabbit	10	120	6	69d,e
	10	150-250	5	104t
Cat	10	100	10	69d,e
Dog	10	100-150	8	69d,e
Monkey	10	150	5	69d,e

this volume. The fluorophosphates are considerably less toxic, although the potency of PF-3 and di-sec-butyl fluorophosphate for the monkey approaches that of the Trilons. The limited data for MFI indi-

TABLE 8. LD_{50} 's of isopropyl methanefluorophosphate (MFI).

The figures in parentheses are the number of animals used.

Route of administration	Species	Approximate LD_{50} (mg/kg)		Reference
Intravenous	Rat	0.045	(30)	66i
	Rabbit	0.016	(44)	66i
Percutaneous	Mouse	1.08	(40)	69d
<i>Per os</i>	Rabbit	0.925	(19)	66i
	Rat	0.55	(66)	66i

TABLE 9. Toxicity of isopropyl ethanefluorophosphate by inhalation.

The animals were totally exposed to the vapor of the agent. All concentrations were nominal.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³)	Number of animals	Reference
Mouse*	5	245	80	69e
	10	330	120	69d,e
	10	350-1,000	8	104t
	30	570	60	69e
Rat	10	260	6	69e
	10	<350	4	104t
Guinea pig	10	>210	6	69e
	10	350-1,000	4	104t
Rabbit	10	230	4	69e
	10	350-1,000	4	104t
Cat	10	170	6	69e
Dog	10	230	4	69e
Monkey	10	210	3	69e

* Subcutaneous LD_{50} (8 mice) = approx. 0.4 mg/kg.^{104t} Percutaneous LD_{50} (40 shaved mice) = 1.7 mg/kg.^{69d}

cate that the species variation in susceptibility is not pronounced. On the other hand the animal species exhibit considerable variation when MCE, and particularly the fluorophosphates, come into consideration.^d This variation makes estimates of the human lethal dosage precarious. In the case of PF-3 comparison of the systemic effects produced upon exposure of human subjects (see preceding section) and of monkeys^{261,p,r,63,64,83} to dosages in the order of 300 to 400 mg min/m³ indicate clearly that man is the more resistant species. Man was affected but not prostrated, whereas the monkeys were severely prostrated and some were killed. How much more resistant man is to PF-3 than the monkey is not known, nor does the same relationship necessarily hold for the Trilons.

^d The high $L(Ct)_{50}$'s of PF-1 and PF-3 (possibly also MCE) for the rabbit are due largely to inhibition of respiration. When inhibitory respiratory reflexes are suppressed, or when the agents are injected, the rabbit is not found to be excessively resistant.^{26g,i,j,53}

TABLE 10. Toxicity of dimethyl fluorophosphate (PF-1) by inhalation.

The animals were totally exposed to the vapor of the agent. All concentrations were nominal. Injection and percutaneous toxicity figures are given in the footnotes. Except for the mouse $L(Ct)_{50}$'s, the figures are very rough approximations based on only a few animals of each species.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³)	Reference
Mouse*	1	1,200	26l
	2	1,740	26h
	10	2,550	11
	10	3,000	49
	30	5,000 ±	26f
	120	>5,000	26f
Rat	1	1,800 ±	26f,l
	10	3,000-6,000	26a, 104b
Guinea pig	1	7,000 ±	26f,l
	0.5-4	8,000 ±	26f
Rabbit†	1	>12,000	26f, 104b
Cat‡	1	6,000 ±	26l
Dog§	1	6,000 ±	26l
Goat	3-5	20,000 ±	26f
House fly	10	≤30	26l
Mosquito	10	≤30	26l

* Mouse intravenous LD_{50} = 0.45 mg/kg.^{26g} Mouse intraperitoneal LD_{50} = 3-4 mg/kg.^{26g} Mouse percutaneous LD_{50} (70 mice) = 0.72 mg/animal, or approx. 36 mg/kg.⁴⁹

† Rabbit intravenous LD_{50} = 2-4 mg/kg.^{26g}

‡ Cat intravenous LD_{50} = 1.5 mg/kg.²⁶ⁱ

§ Dog intravenous LD_{50} = 1-2 mg/kg.^{26g}

|| *Aedes aegypti*.

TABLE 11. Toxicity of diethyl fluorophosphate by inhalation.

The animals were totally exposed to the vapor of the agent. All exposure times were for 10 minutes and all concentrations were nominal. Except for the mouse $L(Ct)_{50}$'s, the figures are very rough approximations based on 6 to 18 animals per species.

Species	$L(Ct)_{50}$ (mg min/m ³)	Reference
Mouse*	8,200	11
	4,100	49
	4,000-6,000	104b
Rat	7,000-14,000	26b, 104b
Guinea pig	7,000-14,000	26b, 104b
Rabbit	>14,000	104b

* Mouse percutaneous LD_{50} (60 mice) = 0.70 mg/animal, or approx. 35 mg/kg.⁴⁹

As indicated by the data of the toxicity tables, the "rate of detoxification" as measured by the increase of the lethal dose with increase of time of its administration, is marked for PF-1, moderate for MFI and isopropyl ethanefluorophosphonate, and slight but definite for MCE and PF-3. More detailed data bearing on the rate of detoxification as determined

TABLE 12. Toxicity of diisopropyl fluorophosphate (PF-3) by inhalation.

The animals were totally exposed to the vapor of the agent. All concentrations were nominal. Except for the mouse and rat $L(Ct)_{50}$'s, the figures are rough approximations based on relatively few animals per species. A total of 39 monkeys were exposed.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³)	Reference
Mouse	1	4,000	104e
	2	3,800	104e
	5	2,700	104e
	10	3,500	104e
	10	5,500	49
	10	5,900	26c,p
	30	4,500	104e
	100	>6,400	26p
Rat	1	4,200	104h
	2	3,600	104h
	5	2,850	104h
	10	2,800	104h
	30	4,500	104h
Guinea pig	10	>8,200	104b
Rabbit	10	(8,000 ±)	104b
Dog	10	5,000 ±	83
Goat	10	6,000-7,000	83
Monkey	2	500-800	26l
	2-15	500 ±	63, 64
	10	800 ±	83
	100	1,000-2,000	26p,r

TABLE 13. LD_{50} 's of diisopropyl fluorophosphate (PF-3).

Route of administration	Species	Approximate LD_{50} (mg/kg)	Reference
Intravenous	Rabbit	0.3-0.4	67b
		0.4 ±	53
		0.5-0.75	32a
	Cat	<3	33a
	Goat	0.8 ±	53
Intramuscular	Monkey	0.1-0.2	48, 66a, 67f
	Rat	2-	32b
	Rabbit	0.75-1.0	67b
Subcutaneous	Mouse	4 ±	86, 104e
	Rat	3 ±	86
	Rabbit	1 ±	86
	Dog	3 ±	86
	Goat	1 ±	86
Percutaneous	Mouse	72 ± (1.45 mg/mouse)	49
<i>Per os</i>	Mouse	36.8	68a
		2 ±	86
		5-10	26r
	Rat	6 ±	86
By eye	Rabbit	9.8	68b
	Rabbit	1.4	32a

by inhalation and injection experiments will be found in the references cited in the tables. Other references are also pertinent.^{26f,g,43,53,55}

Both the Trilons and the fluorophosphates are

TABLE 14. Toxicity of di-*sec*-butyl fluorophosphate by inhalation.

The animals were totally exposed to the vapor of the agent. All concentrations were nominal. Except for the mouse $L(Ct)_{50}$'s, the figures are very rough approximations based on 2 to 23 animals of each species.

Species	Exposure time (min)	$L(Ct)_{50}$ (mg min/m ³)	Reference
Mouse	10	5,140	26r
	10	5,400	104i
Rat	10	4,000–10,000	26r, 104i
Guinea pig	10	>18,000	26r, 104i
Rabbit	10	5,000–10,000	26r, 104i
Cat	10	6,000 ±	26r
Dog	10	4,000–6,000	26r
Monkey	2	100–400	26q,r

TABLE 15. Toxicity of dicyclohexyl fluorophosphate by inhalation.

The animals were totally exposed to the vapor of the agent. All exposures were for 10 minutes and all concentrations were nominal. Except for the mouse $L(Ct)_{50}$'s, the figures are very rough approximations based on 2 to 20 animals per species.

Species	$L(Ct)_{50}$ (mg min/m ³)	Reference
Mouse	800	104l
	1,100	26n
Rat	1,200 ±	26n, 104l
Guinea pig	6,000–10,000	104l
Rabbit	1,200–2,800	26n, 104l
Dog	1,000–1,400	26l, 26n

TABLE 16. Toxicity of *bis*(dimethylamido)phosphoryl fluoride.

Species	Approximate $L(Ct)_{50}$ (mg min/m ³)		Approximate LD_{50} (mg/kg)			
	Nominal, $t = 10$ min	Reference	Intravenous	Subcutaneous	<i>Per os</i>	Reference
Mouse	950	26j	...	1	2 ±	86, 104o
Rat	2,000–4,000	26j, 104o	...	0.3–0.4	1 ±	86
Guinea pig	>4,000	26j, 104o	...	2 ±	4 ±	86
Rabbit	>2,000	104o	3 ±	6 ±	3 ±	86, 104o
Cat		...	...	...	2 ±	86
Goat		...	...	2 ±	...	86
Monkey		...	...	...	>1 or 2	86

“quick-kill” agents. Although occasional deaths are delayed for 1 or 2 days, most lethally poisoned animals die within 2 hours after exposure, and the majority during or within a few minutes after exposure. Detailed statements may be found in the references cited in the toxicity tables. A special study has shown that PF-1 and PF-3 are only slightly slower in speed of action than hydrogen cyanide (AC), although the lower volatilities of the fluorophosphates would make high concentrations relatively difficult to attain in the field.⁵⁵

SYMPTOMS AND PATHOLOGY

The symptoms produced by exposure to the Trilons and fluorophosphates are those which characterize the nicotinic and muscarinic actions of parasympathomimetic agents in general. There are also evidences of central nervous stimulation. Although there are variations according to species, agent, and dosage, frequent mention has been made of the following: lacrimation and salivation; apprehension; coughing, dyspnea, and gasping; hyperexcitability, incoordination, and ataxia; tremor, muscular twitchings, and convulsions; sometimes bronchospasm, pilomotor stimulation, urination, and defecation;

general weakness and depression; and finally cessation of respiration. Detailed descriptions for the various agents and species may be found in the references cited in the toxicity tables (see also the references given under the section “Protection and Treatment”).

Respiratory failure is probably the usual primary cause of death.⁸³ However, the action of the agents as revealed, for instance, by a study of PF-3,^{33a} clearly involves most of the important systems in the body and the weakest link in the chain of events leading to death is questionable. One point of view is that bronchospasm may be important in some species, including man. This is not true in the cat.³³ Because of the early time of death, pathological changes frequently are not conspicuous at autopsy.^{26b,g,83,87,104} Dicyclohexyl fluorophosphate, which seems to act somewhat more slowly than the other fluorophosphates, has been found to produce in rabbits a marked pulmonary edema and edema of the perivascular connective tissue, marked pulmonary hyperemia, large areas of atelectasis, hepatic congestion and incipient central atrophy, and slight lymphorhexis.^{26m} The action of *bis*(dimethylamido)phosphoryl fluoride is slower still and the pathologi-

TABLE 17. Toxicity of vapors through the skin (body only exposures).

Agent	Species	Exposure time (min)	Dosage (<i>Ct</i> in mg min/m ³)		Mortality	Reference	
			Nominal conc.	Analytical conc.			
Dimethyl fluorophosphate (PF-1)	Mouse	10	124,000	122,000	6/6	26d	
		10	51,600	48,200	3/6	26d	
		15	13,000	12,600	0/6	26d	
Ethyl dimethylamidocyanophosphate (MCE)	Mouse	10	3,850	2,500	<i>L(Ct)</i> ₅₀	69c, d	
		10	1,000	750*	<i>L(Ct)</i> ₅₀	69c	
		60	6,060	3,900	<i>L(Ct)</i> ₅₀	69d	
	Guinea pig	16	11,200	6,100	0/6	69c	
		Dog	16	11,200	6,100	0/1	69c
	210		45,400	29,000	0/1	69d	
	227-360		...	80,000 ±	<i>L(Ct)</i> ₅₀ (8 animals)	66k	
	Rabbit	77-282	...	19,000 ±	<i>L(Ct)</i> ₅₀ (36 animals)	66h	
	Isopropyl methanefluorophosphonate MFI <i>bis</i> (β-Chloroethyl) sulfide (H)	Mouse	16	8,720	...	1/20	69e
Mouse		10	...	3,500	<i>L(Ct)</i> ₅₀	15	
Rabbit		13.5	...	2,900	0/1	15	
		18	...	4,000	0/1	...	
		32	...	5,800	1/1	...	
		35	...	8,000	1/1	...	
		60	...	13,400	0/1	...	
		80	...	20,500	1/1	...	
		Dog	60	...	6,550	0/1	15
60			...	9,600	1/1	...	
30			...	15,400	1/1	...	
60			...	17,600	1/1	...	
60			...	24,600	1/1	...	
<i>tris</i> (β-Chloroethyl)amine (HN3)		Mouse	10	...	860	<i>L(Ct)</i> ₅₀	15
		Rabbit	47-140	...	>5,500	<i>L(Ct)</i> ₅₀	26t,u
	Dog	30	...	13,300	0/1	15	
		45	...	14,500	0/1	...	
		75	...	21,400	1/1	...	
		100	...	51,600	1/1	...	

* Skin of mice shaved.

cal changes are somewhat different. Attention has been directed to marked pleural effusion, pulmonary edema and hyperemia, and inflammation of the submucosal layer of the tracheal and broncheal epithelium.^{26j,k,86} The references cited in the toxicity tables should be consulted for more detailed pathological information.

EFFECTS ON AND THROUGH THE SKIN

Neither the Trilons nor the fluorophosphates exert a vesicant action.^{11,26d,53,66h,k,69c,d,e,87,90} With regard to absorption of the agents through the skin, the data given in Table 17 suggest that vapor dosages reasonably attainable in the field would not produce significantly severe systemic effects percutaneously in the cases of the larger animals nor, presumably, in man. Moreover, ordinary clothing can be expected to afford some protection against the vapors; CC-2 impregnated clothing, considerable protection; and carbon clothing, virtually complete protection.^{69d,e} On the other hand, liquid contamination of the skin

with MCE or MFI is potentially very dangerous (see Tables 6 and 8).^{66h,i} In the case of MCE rapid removal of the liquid by blotting is effective treatment. Apparently chloramides do not react readily with MCE. Consequently antigas ointments are of limited value except in so far as their application can facilitate the removal of the agent by solvent or mechanical action. In experiments with rabbits it was found that interposition of a single layer of plain herringbone twill increased by 6- to 8-fold the dose of MCE that must be applied to the skin to cause death. A single layer of CC-2 impregnated cloth increased the dose 10- to 12-fold; two layers of this cloth, 20-fold; and one layer of carbon cloth, 15-fold.^{66h,i} Thus clothing, particularly protective clothing, is of considerable value in preventing the absorption of lethal doses of this agent.

PROTECTION AND TREATMENT

Numerous substances and procedures for prophylaxis and therapy of the systemic effects of fluoro-

phosphate and Trilon poisoning have been investigated.^{26f,g,h,i,32,33a,44,66a,b,g,h,i,k,67a,b,c,e,f,69e,h,104h,n,s} Of these the injection of atropine and magnesium sulfate seems to offer the most promise and has been recommended for use in the event of human poisoning.^{66i,67e} These therapeutic agents suppress the autonomic symptoms. Injection of Nembutal in addition will control the convulsions which occur in MCE poisoning.^{66h,87} However, in severe poisoning the action of these drugs will merely delay but not prevent death. In any event it is essential that therapy be instituted promptly. Adequacy of protection by the gas mask has been mentioned, as have been methods of treating eye effects and of preventing percutaneous absorption of liquid contamination.

PHYSIOLOGICAL MECHANISM

In 1941 and 1942 British workers reported that the dialkyl fluorophosphates are very potent inhibitors of cholinesterase.^{103,104a,h} Since that time extensive studies have been made on the clinical pathology and biochemistry of action of these compounds,^{26e,g,33,43-49,53,54,66,67} and more recently of the Trilons, which have also proved to be potent anticholinesterases.^{66g,h,j,67,69e,f,h,i} The results have already begun to appear in the open literature and need not be reviewed here. It is obvious that the agents will be of great value as tools in physiological and biochemical research. Their possible use in the treatment of myasthenia gravis has also been under investigation.

Chapter 10

METHYL FLUOROACETATE AND RELATED COMPOUNDS^a

By Birdsey Renshaw and Marshall Gates

10.1

INTRODUCTION

REPORTS THAT methyl fluoroacetate is highly toxic were received from Polish investigators by the British in 1942 and prompted extensive studies in the United Kingdom and United States. Fluoroacetic acid and many simple derivatives including salts and esters, β -fluoroethanol and its esters, and salts and esters of γ -fluorobutyric acid, γ -fluoro- β -hydroxybutyric acid, and γ -fluorocrotonic acid, proved to be highly toxic by inhalation, injection, and ingestion. These compounds produce death, usually after a latency of one-half to several hours, by action on the heart or central nervous system.

Compounds of this group are not seriously considered for large-scale use in chemical warfare at the present time because: (1) although very toxic for some species, the human lethal and incapacitating doses are believed to be comparable to, or considerably greater than, those of the currently standardized persistent and nonpersistent agents; (2) the stable derivatives do not possess sufficiently high vapor pressures to be dispersed from available munitions in high concentrations as nonpersistent agents; and (3) the gas mask affords adequate protection.

The salts of fluoroacetic and related toxic acids are nonvolatile, stable in aqueous solution, and approximately as toxic when administered orally as when injected. They are, therefore, potential water poisons in warfare and are proving to be highly effective bait poisons for rodents.

^a Based on information available to Division 9 of the National Defense Research Committee [NDRC] as of October 1, 1945.

Attention is directed to a recent paper by J. S. C. Marais, entitled *Monofluoroacetic Acid, The Toxic Principle of "Gifblaar" Dichapetalum cymosum (Hook) Engl.*, Onderstepoort Journal of Veterinary Science and Animal Industry **20**, 67-73 (1944). The early Dutch settlers in South Africa gave the name "Gifblaar" to a plant the leaves of which are poisonous to livestock. A number of toxicological and chemical studies have been made in South Africa since about 1900, and it is apparently a remarkable coincidence that the active principle was being identified there at the same time that fluoroacetic acid derivatives were being actively studied as potential chemical warfare agents in the United Kingdom and United States. Although the South African literature corroborates many of the chemical and toxicological findings summarized in this chapter, a cursory survey fails to reveal data permitting an independent estimation of the human lethal dose.

The compounds of this group selectively poison enzyme systems and as inhibitors will be of value in the study of intermediary metabolism.

10.2

Synthesis and Properties

Approximately 160 aliphatic fluorine compounds have been prepared by NDRC and British investigators for evaluation as chemical warfare agents. The compounds, their physical properties, and references to their synthesis and toxicity are listed in Table 1.

10.2.1

Synthesis

In general, the syntheses have been effected by the fluorination of corresponding chlorine and bromine compounds by treatment with metallic fluorides, usually anhydrous potassium fluoride, less frequently silver fluoride, mercuric fluoride, or antimony fluoride, according to known procedures.^{19,22,36,92,101,103,104}

Application to the fluorinated compounds of standard synthetic methods has resulted in a variety of derivatives including representatives of most of the common aliphatic types.

The methods may be illustrated by the following examples.

1. *Methyl fluoroacetate*^{19,36a,90,92a} has been prepared on a large laboratory scale (50 lb) by heating methyl chloroacetate under pressure with anhydrous potassium fluoride at 220 C for 5 hours. The product is distilled directly from the pressure vessel and is purified by fractionation. Yields in the neighborhood of 75-77 per cent are obtained.

The compound is a colorless mobile liquid with a faint ester-like odor. Unlike the other haloacetates, it has no lacrimatory properties. It boils at 104.5 C, freezes at -35 C, and is soluble in water to the extent of about 15 per cent. Its physical properties have been thoroughly investigated.^{14,87,95a}

2. *Sodium fluoroacetate*,^{19,22,92e} because of its promise as a rodenticide, has been prepared on a much larger scale than has any other member of the fluoroacetate series. Complete pilot plant conditions were worked out during the course of preparing 1,000 lb²² for use in experimental rodent-control projects. It is prepared by saponification of ethyl fluoroacetate

TABLE 1. Aliphatic fluorine compounds examined as candidate chemical warfare agents.

The compounds are arranged in three major categories in the following sequence: (1) compounds containing not more than one fluorine atom attached to any carbon atom; (2) compounds containing two fluorine atoms attached to any one carbon atom; and (3) compounds containing three fluorine atoms attached to the same carbon atom. Within each major category compounds are arranged in sequence according to the following types: hydrocarbons, alcohol derivatives, amines, carbonyl derivatives, and acid derivatives.

The following abbreviations are used: n_D^t , refractive index at t C; d^t , density in g/ml at t C; $d_{t_2}^{t_1}$, specific gravity at t_1 C in reference to water at t_2 C; mp, melting point in C; bp^p, boiling point in C at p mm Hg; vp^t, vapor pressure in mm Hg at t C; and vol^t, saturation concentration (volatility) in mg/l at t C.

Centigrade scale is used throughout the table.

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
1. 1-Chloro-2-fluoroethane	19, 92e	bp ⁷⁵⁰	52–53°	19	13, 91f
2. 1-Bromo-2-fluoroethane	92g	bp ⁷⁶⁰	71–73°	31c	13
3. <i>sym</i> -Dichlorodifluorodinitroethane	...	d^{25}	1.708	52	52
	...	mp	12–18°	52	...
	...	bp ³⁵	55–56°	52	...
	...	vol ²⁵	70.1	52	...
4. <i>tert</i> -Butyl fluoride	3, 19	bp ⁷⁶⁰	14.5–16°	3	13
5. β -Fluoroethanol	19, 92c	n_D^{20}	1.3618	14	13, 91c, 92c
	...	d^{20}	1.0913	14	...
	...	bp ⁷⁴⁵	99–100°	14	...
	...	vol ²⁵	74.8	14	...
6. Methyl β -fluoroethyl ether	91f	bp	ca. 60°	91f	91f
7. Chloromethyl β -fluoroethyl ether	19	bp ²⁵	35–40°	19	13
8. β -Chloroethyl β -fluoroethyl ether	19	bp ²⁰	55–58°	19	13
9. β -Fluoroethyl β -hydroxyethyl ether	19	n_D^{26}	1.4050	19	13
	...	bp ⁷	61–62°	19	...
10. Methyl β -fluoroethoxyacetate	19	bp ¹⁵	85–88°	19	...
11. β -Fluoroethyl phenyl ether	92f	mp	41	92f	91h
	...	bp ¹⁷	92.5°	92f	...
12. 2'-Fluoro-2,4-dinitrophenetole	19	mp	89–91°	19	13
13. β -Fluoroethyl β -naphthyl ether	92g	mp	49.5–50°	92g	91h
14. <i>bis</i> (β -Fluoroethyl)formal	19	n_D^{20}	1.3809	14	13
	...	bp ³⁰	75–76°	14	...
	...	vol ²⁵	10.23	14	...
15. <i>tris</i> (β -Fluoroethyl)orthoformate	19	n_D^{20}	1.3946	14	13
	...	d^{20}	1.2316	14	...
	...	bp ^{20–21}	126–128°	14	...
	...	vol ²⁵	0.330	14	...
16. β -Fluoroethyl acetate	92e	bp	118–119°	92e	91f
17. β -Fluoroethyl chloroacetate	92g	bp ¹⁵	78–79°	92g	91h
	...	bp ⁷⁶⁰	178°	92g	...
18. β -Fluoroethyl trichloroacetate	19	bp ¹⁵	84–87°	19	13
19. β -Fluoroethyl propiolate	25	n_D^{29}	1.4340	25	20
	...	d^{29}	1.2465	25	...
20. β -Fluoroethyl ϵ -bromocaproate	92k	bp ¹³	142°	92k	92k
21. <i>bis</i> (β -Fluoroethyl) acetylenedicarboxylate	19	mp	54–55°	19	13
22. <i>bis</i> (β -Fluoroethyl) chloromaleate (or fumarate)	19	bp ¹	125–127°	19	13
23. β -Fluoroethyl N-methylcarbamate	19, 30	bp ²⁰	91–94°	19	...
24. β -Fluoroethyl N-nitroso-N-methylcarbamate	19, 30	bp ¹⁵	70–85°	19	13
25. β -Fluoroethyl N-(β -chloroethyl)carbamate	19, 30	bp ¹	105–108°	19	...
26. β -Fluoroethyl N-nitroso-N-(β -chloroethyl)carbamate	19, 30	bp ²	118–121°	19	13
27. β -Fluoroethyl glycine hydrochloride	92j	mp	150.5°	91l	91l
28. β -Fluoroethyl betaine hydrochloride	92j	mp	122°	91l	91l
29. β -Fluoroethyl nitrite	19	n_D^{20}	1.3589	14	13
	...	d^{20}	1.1427	14	...
	...	bp ³⁸⁰	42–45°	14	...
	...	bp ⁷⁶⁰	58–60°	14	...
	...	vol ²⁵	664	14	...
30. β -Fluoroethyl thiocyanate	92g	bp ¹⁹	77.5–78.5°	92g	91h
31. β -Fluoroethyl xanthate	92g	bp	208–210°	92g	91h
32. β -Fluoroethyl chlorocarbonate	19, 30	d^{20}	1.3995	14	13
	...	bp ⁷⁵⁰	128°	14	...
	...	vol ²⁵	47.0	14	...

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
33. <i>bis</i> (β -Fluoroethyl) carbonate	19	d^{20}	1.2939	14	13
	...	bp ⁴	71–72°	14	...
	...	vol ²⁵	1.15	14	...
34. β -Fluoroethyl chlorosulfonate	19, 92g	bp ²⁰	85–86°	19	13, 91f
35. <i>bis</i> (β -Fluoroethyl) sulfate	19, 92g	n_D^{20}	1.4177	14	13, 91f
	...	d^{25}	1.3678	14	...
	...	bp ²	80–81°	14	...
	...	vol ²⁵	0.425	14	...
36. <i>tris</i> (β -Fluoroethyl) arsenite	19	bp ^{2.5}	132–134°	19	13
37. <i>tetrakis</i> (β -Fluoroethyl) silicate	19	bp ^{0.5}	102–104.5°	19	13
38. <i>tris</i> (β -Fluoroethyl) borate	...	bp	192°	91o	91o
39. β -Fluoroethoxydichlorophosphine	19, 31c	bp ³⁰	50°	31c	13
	...	bp ⁷⁶⁰	140–145°	31c	...
40. <i>bis</i> (β -Fluoroethyl) hydrogen phosphite	92n	bp ^{1.7}	109–110°	92n	91h
41. <i>bis</i> (Diethylamino)- β -fluoroethoxyphosphine	19	bp ²⁵	108–111°	19	13
42. Ethyl- <i>bis</i> (β -fluoroethoxy) phosphine	19	bp ^{0.5}	40–49°	19	13
43. <i>tris</i> (β -Fluoroethyl) phosphite	19	bp ^{0.5}	100–105°	19	13
44. <i>bis</i> (β -Fluoroethyl) fluorophosphate	92m	bp ^{0.5}	90–95°	31b	91f
45. <i>bis</i> (β -Fluoroethyl)phosphoryl chloride	19	bp ^{0.5}	108–112°	19	...
46. β -Fluoroethylmercaptan	33b	bp ²²⁵	38–39°	33a	13
47. <i>bis</i> (β -Fluoroethyl) sulfide	48	vp ^{25b}	2.67	48	48
48. β -Chloroethyl β -fluoroethyl sulfide	19	n_D^{20}	1.4872	19	13
	...	d^{20}	1.228	19	...
	...	bp ²⁰	91.5°	19	...
49. β -Fluoroethyl thiolacetate	33b	n_D^{20}	1.4538	14	13
	...	d^{20}	1.1451	14	...
	...	bp ³⁰	58–59°	14	...
	...	vol ²⁵	28.6	14	...
50. 1,2- <i>bis</i> (β -Fluoroethylthio)ethane	92h	bp ¹⁷	138–139°	92h	91l
51. γ -Fluoropropanol	31d	n_D^{20}	1.3819	31d	20, 59c
	...	bp	125–128°	31d	...
52. Epifluorohydrin	19	n_D^{20}	1.3696	14	13
	...	d^{20}	1.0967	14	...
	...	bp ⁷⁵³	83.5–84°	14	...
	...	vol ²⁵	260	14	...
53. Epifluorohydrin-phosphorus trichloride	19	bp ¹²	72–74°	19	13
54. 1-Chloro-3-fluoropropanol-2	19	n_D^{20}	1.4290	14	13
	...	d^{20}	1.3014	14	...
	...	bp ⁵⁰	75–76°	14	...
	...	vol ²⁵	13.03	14	...
55. 2-Chloro-3-fluoro-1-methoxypropane	19	bp ⁷⁵⁰	121–123°	19	20
56. 1-Fluoro-2-hydroxy-3-methoxypropane	19	bp ³⁵	70°	19	13
57. 1-Dimethylamino-2-hydroxy-3-fluoropropane	19	bp ⁵⁰	82–83°	19	13
58. 3-Fluoro-2-hydroxypropane-1-sulfonic acid, sodium salt	19	...		...	20
59. β -Fluoro- β' -methoxyisopropyl chlorosulfinate	19	bp ²²	93–96°	19	13
60. γ -Fluoro- β -hydroxypropyl propyl sulfide	19	bp ²²	105–107°	19	13
61. <i>bis</i> (β -Fluoroethyl)amine	94	bp ⁷⁶⁴	123–126°	94	91p
62. <i>bis</i> (β -Fluoroethyl)methylamine	...	bp ⁷⁶²	123–124°	91p	91p
63. β -Fluoroethyltrimethylammonium bromide	92i	mp	244°	92i	91l
64. β -Fluoroethyltriethylammonium bromide	92i	mp	237°	92i	91h
65. N- β -Fluoroethylpyridinium bromide	92i	mp	180°	92i	91h
66. Fluoroacetaldehyde	92l	bp	89–92°	91o	91o, 92l
67. Fluoroacetaldehyde 2,4-dinitrophenylhydrazone	...	mp	147°	91o	91o
68. Fluoroacetodiazomethane	19	bp ¹⁵	41–44°	19	13
69. ω -Fluoroacetophenone	19	n_D^{20}	1.5309	14	13
	...	d^{20}	1.1747	14	...
	...	mp	22.8°	14	...
	...	bp ³	79–80°	14	...
	...	vol ²⁵	0.591	14	...
70. Fluoroacetic acid	92b	mp	31°	92b	91c
71. Sodium fluoroacetate	19, 22, 92e	...		...	20

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
72. Aluminum fluoroacetate	56	...		...	59d
73. Cupric fluoroacetate	56	...		...	59d
74. Mercuric fluoroacetate	56	...		...	59d
75. Thallous fluoroacetate	56	...		...	59d
76. Triethyllead fluoroacetate	92f	mp	180.5°	92f	91h
77. Methyl fluoroacetate	19, 92a	n_D^{20}	1.3679	14	13, 20, 91c
	...	d^{23}	1.0593	14	...
	...	mp	35°	14	...
	...	bp ⁷⁵²	103–103.5°	14	...
	...	vol ²⁵	119	14	...
78. Ethyl fluoroacetate	19, 92e	n_D^{20}	1.3759	14, 24q	13, 91c
	...	d^{25}	1.0826	14, 24q	...
	...	bp ⁷⁵⁰	114–118°	14, 24q	...
	...	vol ²⁵	68.57	14, 24q	...
79. Ethyl dichlorofluoroacetate	47b	bp ⁷³⁰	131.5–132°	47b	13
80. β -Fluoroethyl fluoroacetate	19, 92d	n_D^{20}	1.3802	14, 19	13, 91d
	...	bp ⁴⁸	85°	14, 19	...
	...	vol ²⁵	7.81	14, 19	...
81. β -Chloroethyl fluoroacetate	19, 92d	d^{20}	1.3160	14	13, 91c
	...	bp ²³	86°	14	...
	...	vol ²⁵	3.55	14	...
82. Allyl fluoroacetate	19, 92l	n_D^{20}	1.4063	14	13, 91l
	...	d^{20}	1.0961	14	...
	...	bp ⁵⁰	64.5–65°	14	...
	...	vol ²⁵	31.69	14	...
83. Propyl fluoroacetate	92e	bp	135–137°	92e	91c
84. Isopropyl fluoroacetate	19, 92e	n_D^{20}	1.3804	14	13, 91c
	...	d^{20}	1.033	14	...
	...	bp ⁷⁵²	121–123°	14	...
	...	vol ²⁵	62.31	14	...
85. β -Ethylhexyl fluoroacetate	...	...		...	59a, 59c
86. Phenyl fluoroacetate	19	mp	61.5–63°	19	13
87. <i>p</i> -Chlorophenyl fluoroacetate	19	mp	52–54°	19	13
88. Cholesteryl fluoroacetate	92f	mp	144–144.5°	92f	91h
89. Methylene- <i>bis</i> (monofluoroacetate)	92l	mp	57°	92l	92l
90. Glycol <i>bis</i> (monofluoroacetate)	92f	bp ¹⁷	140–141°	92f	13, 91h
91. Fluoroacetylcholine chloride	...	...		...	...
92. Fluoroacetylsalicylic acid	19, 92j	mp	141–144°	19, 92f	13, 91h
	...	mp	131.6°	...	...
93. S- β -chloroethyl fluorothiolacetate	19, 92j	bp ¹⁰	80–81°	19	13, 91l
94. Phenyl fluorothiolacetate	92f	mp	36.5–37.5°	92f	91h
	...	bp ¹⁸	132°	92f	...
95. Methyl fluoroselenolacetate	11	n_D^{20}	1.4879	14	13
	...	d^{20}	1.573	14	...
	...	bp ⁷⁴²	130–132°	14	...
	...	vol ²⁵	69.95	14	...
96. Fluoroacetyl fluoride	19, 92e	bp ⁷⁶⁰	35–40°	19, 92e	13, 91f
97. Fluoroacetyl chloride	19, 92b	n_D^{20}	1.3831	14	13, 91c
	...	d^{20}	1.3530	14	...
	...	bp ⁷⁵⁰	69–71°	14	...
	...	vol ²⁵	607	14	...
98. Fluoroacetonitrile	19, 92g	n_D^{20}	1.3324	14	13
	...	bp ⁷⁵²	78°	14	...
	...	vol ²⁵	260	14	...
99. Fluoroacetyl isothiocyanate	19	n_D^{20}	1.5327	14	13
	...	d^{20}	1.3527	14	..
	...	bp ⁵⁰	76°	14	..
	...	vol ²⁵	15.51	14	...
100. Fluoroacetic anhydride	92e	bp ¹²	88–89.5°	92e	91c
101. Fluoroacetamide	19, 92b	mp	108°	19, 92b	91c
102. N-Methylfluoroacetamide	92b	mp	64°	92b	...
103. N-Nitroso-N-methylfluoroacetamide	92e	bp ¹⁴	84°	92e	91c

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
104. N- β -Chloroethylfluoroacetamide	19, 92e	mp	65°	19, 92e	13
	...	bp ^{0.3}	77°	19, 92e	...
105. N- β -Hydroxyethylfluoroacetamide	92e	mp	ca. 21°	92e	...
	...	bp ^{0.1}	114°	92e	...
106. N,N-Diethylfluoroacetamide	19	bp ¹²	86°	19	13
107. N,N-bis(β -Chloroethyl)fluoroacetamide	92e	mp	64.5°	31a, 92e	...
	...	bp ^{0.04}	102°	31a, 92e	...
108. α -Fluoroacetanilide	23	mp	73-74°	23	20
109. Fluoroacetyl glycine ethyl ester	92f	mp	50-50.5°	92f	91h
110. Fluoromethylfluoroacetylurea	92l	mp	84°	92l	92l
111. 2-Fluoroethane-1-sulfonyl chloride	92g	bp ¹³	81.5-84.5°	92g	91h
112. Methyl α -fluoropropionate	92e	bp	106.5-108.5°	92e	91f
113. Ethyl β -fluoropropionate	19	bp ⁶⁶	65-68°	19	20
114. Diethyl fluoromalonate	91c	bp ¹³	84-86°	91c	91c
115. Sodium γ -fluorobutyrate	19	...		...	13
116. Methyl γ -fluorobutyrate	10, 19	n_D^{20}	1.3887	10	13
	...	d^{20}	1.0662	10	...
	...	bp ¹⁰⁰	79°	10	...
	...	vol ²⁵	39.6	10	...
117. Methyl α -fluoroisobutyrate	92e	bp	108-109°	92e	91c
118. Ethyl α -fluorobutyrate	47b	bp ⁴⁰	62.5-64°	47b	13
119. Ethyl γ -fluorobutyrate	47b	bp ⁴⁰	68-70°	47b	13
120. β -Fluoroethyl γ -fluorobutyrate	10, 19	n_D^{20}	1.3953	10	13
	...	d^{20}	1.604	10	...
	...	bp ⁷	69°	10	...
	...	bp ²⁵	90°	10	...
	...	vol ²⁵	4.02	10	...
121. β -Chloroethyl γ -fluorobutyrate	10, 19	n_D^{20}	1.4278	10	13
	...	d^{20}	1.2007	10	...
	...	bp ¹	61°	10	...
	...	bp ⁶	80°	10	...
	...	vol ²⁵	1.18	10	...
122. Isopropyl γ -fluorobutyrate	10, 19	n_D^{20}	1.396	10	13
	...	bp ¹⁰⁰	93°	10	...
	...	vol ²⁵	20	10	...
123. Sodium γ -fluorocrotonate	19	...		...	20
124. Methyl γ -fluorocrotonate	10, 19	n_D^{20}	1.4208	10	13
	...	bp ⁵⁰	70-73°	10	...
	...	bp ⁵⁵	74°	10	...
	...	vol ²⁵	15	10	...
125. Methyl β -chloro- γ -fluorobutyrate	10, 19	n_D^{20}	1.4227	10	13
	...	d^{20}	1.2365	10	...
	...	bp ¹⁸	67-70°	10	...
	...	bp ⁴⁰	92°	10	...
	...	vol ²⁵	6.94	10	...
126. Sodium γ -fluoro- β -hydroxybutyrate	19	...		...	20
127. Methyl γ -fluoro- β -hydroxybutyrate	10, 19	n_D^{20}	1.4184	10	13
	...	bp ¹⁰	90°	10	...
	...	bp ¹⁴	94°	10	...
	...	vol ²⁵	1.15	10	...
128. β -Chloroethyl γ -fluoro- β -hydroxybutyrate	19	n_D^{20}	1.4502	19	20
	...	bp ^{0.4}	100-105°	19	...
129. Methyl γ -fluoro- β -methoxybutyrate	19	bp ¹⁰	55°	19	59b
130. γ -Fluorobutyronitrile	19	d^{20}	1.0034	14	13
	...	bp ¹⁰⁰	98°	14	...
	...	vol ²⁵	12.89	14	...
131. γ -Fluorocrotonitrile	19	bp ⁴⁵	79-81°	19	13
132. γ -Fluoro- β -hydroxybutyronitrile	19	n_D^{22}	1.4232	19	59c
	...	bp ⁸	111-114°	19	...
133. γ -Fluorobutyrylcholine chloride	19	(Hygroscopic solid)		19	13

TABLE 1 (Continued).

Compound	Reference to synthesis	Physical properties		Reference	Reference to toxicity data
		Property			
134. Methyl γ -fluorothiolbutyrate	10, 19	n_D^{20}	1.4587	10	13
	...	d^{20}	1.1135	10	...
	...	bp ⁶	54°	10	...
	...	vol ²⁵	8.44	10	...
135. Methyl γ -fluoro- β -hydroxythiolbutyrate	19	n_D^{20}	1.4872	19	20
	...	bp ^{0.2}	68–71°	19	...
136. Methyl α,γ -difluoroacetoacetate	92e	bp ²⁸	113–115°	92e	91c
137. Dimethyl α,α' -difluorosuccinate	19	bp ^{0.7}	68–70°	19	13
138. Ethyl δ -fluorovalerate	91r	bp ¹⁰	54–58°	91r	91r
139. Ethyl ϵ -fluorocaproate	92k	bp ¹⁴	82–84°	92k	92k
140. β -Fluoroethyl ϵ -fluorocaproate	92k	bp ¹⁴	103–105°	92k	92k
141. Ethyl ω -fluorocaprato	91r	bp ¹⁰	134–136°	91r	91r
142. Ethyl ω -fluorohendecanoate	91r	bp ^{0.2}	82–84°	91r	91r
143. <i>sym</i> -Tetrafluordinitroethane	...	...		...	13
144. 1-Chloro-1,2,2-trifluorodinitroethane	...	d^{25}	1.6595	52	13, 52
	...	bp ⁷⁶⁰	98°(decomposes)	52	...
	...	bp ²²⁵	61–62°	52	...
	...	vol ²⁵	382	52	...
145. Ethyl $\alpha,\alpha,\beta,\beta$ -tetrafluoroethyl ether	...	...		...	13
146. $\alpha,\alpha,\beta,\beta$ -tetrafluoroethyl- β -hydroxyethyl ether	...	...		...	13
147. Cyclohexyl $\alpha,\alpha,\beta,\beta$ -tetrafluoroethyl ether	...	...		...	13
148. Dodecyl $\alpha,\alpha,\beta,\beta$ -tetrafluoroethyl ether	...	...		...	...
149. Difluoroacetic acid	47a	bp ⁷⁶⁰	134.2	102	13
	...	bp ²⁰	67–70°	102	...
150. Methyl difluoroacetate	19	bp	72–78°	14	13
	...	vol ²⁵	361	14	...
151. Difluoroacetonitrile (trimeric)	...	...		...	13
152. Bromotrifluoromethane	46b	...		...	...
153. Trifluoroiodomethane	46b	...		...	13
154. β,β,β -Trifluoroethylamine	...	...		...	13
155. β,β,β -Trifluoroisopropylamine	...	...		...	13
156. Dimethylthallium salt of hexoyltrifluoroacetone	46a	...		...	13
157. Thallous trifluoroacetate	...	...		...	13
158. Diethylthallium trifluoroacetate	4	...		...	...
159. Methyl trifluoroacetate	19	bp	42.5–43°	19	13
160. Trifluoroacetyl chloride	19	bp ⁷⁶⁰	9–11°	19	13
161. Trifluoroacetonitrile	...	...		...	13
162. Thallous <i>m</i> -trifluoromethylbenzoate	26b	...		...	13
163. <i>m</i> -Trifluoromethylphenyldichlorarsine	26a	...		...	13

with an excess of sodium hydroxide in dry methanol. The resulting salt is collected, washed with methanol, dried, and screened. The required ethyl fluoroacetate is prepared in yields as high as 75 per cent from commercially available ethyl chloroacetate by a procedure closely resembling that described above for methyl fluoroacetate. Based on the average pilot plant yield, the intermediates required for the production of 100 lb of sodium fluoroacetate are: ethyl chloroacetate, 250 lb; potassium fluoride, 214 lb; sodium hydroxide, 57 lb; methanol, 380 lb.

3. β -Fluoroethanol^{19,36b,90,92c} has been prepared on a large laboratory scale (50 lb) by heating anhydrous potassium fluoride with ethylene chlorohydrin at 180 C for 4 to 5 hours. Yields of 53 per cent are obtained. Except for the difference in temperature the reaction is carried out as described for methyl fluoro-

acetate. During the heating ethylene oxide is formed almost quantitatively from the ethylene chlorohydrin but appears to be the product of a reversible side reaction. Attempts to produce β -fluoroethanol from ethylene oxide and hydrogen fluoride have been unsuccessful.

β -Fluoroethanol is a colorless liquid with a pleasant alcohol-like odor. It boils at 102.5 C at atmospheric pressure and is completely miscible with water. Several of its physical properties have been determined.^{14,95e}

4. Methyl γ -fluorobutyrate^{10,19} is prepared by treating trimethylenchlorobromide with sodium cyanide to produce a mixture of γ -chloro and γ -bromobutyronitriles. The mixture is then heated under pressure with anhydrous potassium fluoride at 200 C to give γ -fluorobutyronitrile, which is converted to

the corresponding methyl ester by treatment with methanol and acid. The overall yield of methyl γ -fluorobutyrate obtained in preparations on a large laboratory scale has been about 25 per cent for the three-step process.

5. *Methyl γ -fluoro- β -hydroxybutyrate, methyl β -chloro- γ -fluorobutyrate, and methyl γ -fluorocrotonate*^{10,19} are prepared as follows. Epichlorohydrin when heated under pressure at 225 C with potassium fluoride is converted into epifluorohydrin. Treatment of the latter with anhydrous hydrogen cyanide and a small amount of sodium cyanide gives γ -fluoro- β -hydroxybutyronitrile in excellent yield. This intermediate is converted to methyl γ -fluoro- β -hydroxybutyrate by treatment with methanol and acid. Methyl β -chloro- γ -fluorobutyrate is produced by the action of thionyl chloride and pyridine on the hydroxy compound. Methyl γ -fluorocrotonate may then be formed by dehydrohalogenation of the β -chloro compound with triethylamine. Yields are good except in the case of the first step involving the conversion of epichlorohydrin to epifluorohydrin. It has not yet been possible to raise the yield of this step above 40 per cent, although 70 to 74 per cent of the unconverted epichlorohydrin is recovered in a form suitable for re-use. The overall yield based on the amount of epichlorohydrin utilized is 46 per cent for methyl γ -fluoro- β -hydroxybutyrate, 39 per cent for methyl β -chloro- γ -fluorobutyrate, and 33 per cent for methyl γ -fluorocrotonate. All three esters are stable colorless liquids.

An alternative method not requiring high-pressure equipment has been developed for the preparation of methyl γ -fluorocrotonate on a laboratory scale.¹⁹ Methyl γ -bromocrotonate, prepared by bromination of methyl crotonate with N-bromosuccinimide, is refluxed at atmospheric pressure with anhydrous potassium fluoride. The product is slowly distilled from the mixture as the reaction proceeds. Yields of approximately 40 per cent are obtained.

In addition to the synthetic procedures already described, a variety of methods has been used to prepare other fluorinated aliphatic compounds. Mention may be made of the preparation of difluoro- and trifluoroacetic acids by the oxidation of 1,1-dichloro-3,3-difluoropropene and 1,1,2-trichloro-3,3,3-trifluoropropene, respectively^{1,5} (see Chapter 40); the preparation of several ethers of $\alpha,\alpha,\beta,\beta$ -tetrafluoroethanol by the addition of alcohol to tetrafluoroethylene; the synthesis of β -fluoroethyl thiolacetate, from which β -fluoroethylmercaptan may be obtained

by hydrolysis, by the peroxide-catalyzed addition of thiolacetic acid to vinyl fluoride;³³ the synthesis of tetrafluoro-1,2-dinitroethane and chlorotrifluoro-1,2-dinitroethane by the addition of nitrogen tetroxide to the corresponding halogenated olefines; and the synthesis of derivatives of ϵ -fluorocaproic acid from cyclohexanone through ϵ -hydroxycaproic acid and the corresponding bromo-compound, which is treated with silver fluoride.^{92k}

10.2.2 Chemical Properties

Methyl fluoroacetate has been the subject of most of the work on the chemistry of the aliphatic fluorine compounds considered in this chapter. It is readily hydrolyzed to fluoroacetic acid and methyl alcohol, the half life of the ester in water buffered at pH 7 being less than 1 hour.²¹ On the other hand, the fluorine atom can be removed from the molecule only by relatively drastic treatment. No reagent has been found which will bring about rapid replacement at room temperatures. As an example, no fluoride ion is produced by heating methyl fluoroacetate for 5 minutes with 20 per cent alcoholic potassium hydroxide, although more prolonged heating (18 hours on steam bath) does result in the incomplete liberation of fluoride ion.^{95d} Concentrated acids at steam bath temperatures hydrolyze the fluorine atom at unspecified rates.⁸⁰ Under physiological conditions of pH and temperature, no fluoride ion is liberated in 72–96 hours in the presence of any of a variety of nitrogen bases, sulfur compounds, and inorganic salts.²⁸

A further example of the chemical inertness of the fluorine atom in fluoroacetates is given by the following comparison of the rates of replacement of halogen by sulfite in the ethyl esters of fluoroacetic, chloroacetic, and bromoacetic acids:⁹⁹

Compound	Temp C	Bimolecular velocity constant
Ethyl bromoacetate	25	18.3
Ethyl chloroacetate	25	0.13
Ethyl fluoroacetate	45	4.5×10^{-5}

Limited data on the storage stabilities^b of both

^b A recent report from the Chemical Warfare Service (TCIR 345, *Surveillance of Fluorine Compounds*, September 5, 1945) testifies to the stability of methyl fluoroacetate and related compounds with respect to pressure development as follows: (1) methyl fluoroacetate and β -fluoroethanol are stable in 75-mm steel shell with respect to pressure for at least 1 year at 65 C; (2) methyl γ -fluorobutyrate does not develop pressure in 6 months at 65 C when in contact with a steel strip in glass apparatus; and (3) methyl γ -fluoro- β -hydroxybutyrate develops a pressure of about 120 psi at 35 per cent void in glass apparatus at 65 C in either the presence or absence of a steel strip.

methyl fluoroacetate and sodium fluoroacetate also illustrate the high stability of these compounds. Methyl fluoroacetate undergoes no visible change on storage for 8 months at 60 C in glass containers in the presence of varnished steel, but a slight deposit of silica forms in the presence of bare steel.⁸⁶ Sodium fluoroacetate undergoes no visible change, loses no weight, and does not alter in fluoride content on storage for 30 days at 65 C in tin-plated cans; the tin surfaces show no change.^{47c}

Methyl fluoroacetate resists oxidation by aqueous permanganate or chromate. In the presence of chromic acid plus concentrated sulfuric acid, production of hydrogen fluoride occurs, slowly in the cold and rapidly on heating.⁸⁰

Methyl fluoroacetate exhibits a thiosulfate demand on heating at 100 C.²¹

There is no evidence that other members of this series differ strikingly from methyl fluoroacetate in the stability of the fluorine atom, or that they exhibit peculiarities in the reactions of the more common functional groups.

10.2.3 Detection and Analysis

The fluorine atom in compounds of the fluoroacetate type is too stable toward hydrolysis to make practical the use of this reaction for purposes of

identification. Therefore, recourse is had to oxidative or thermal decomposition producing hydrogen fluoride, which is then detected by its etching effect on glass or by its ability to bleach metallic lakes of appropriate dyes.^{17,55,80,81,95b} A device making use of the etching effect to produce a nonwetttable surface in small glass tubes has been examined by the British.^{81,82,83} Hot platinum filaments and hot platinized silica gel both decompose volatile fluorine compounds and both have been utilized in experimental apparatus designed for field use.^{17,55}

Satisfactory tests for fluoroacetate ion in water have been developed.^{49,84}

All quantitative methods for determination of fluorine in compounds of the fluoroacetate type have involved the conversion of the organically bound fluorine to fluoride ion, which is then determined by one of the standard methods.

The detection and analysis of aliphatic fluorine compounds are reviewed in more detail in Chapters 34 and 37.

10.3 CHEMICAL STRUCTURE IN RELATION TO TOXICITY^{24i,44,91b,f,i,r,92e}

In Table 2 are listed representative compounds which do and do not possess to a marked degree the

TABLE 2. Aliphatic fluorine compounds illustrating the relationships between molecular structure and toxicity.

Compounds exhibiting definite fluoroacetate- or γ -fluorobutyrate-like toxicity*	Reference	Compounds exhibiting no or only slight fluoroacetate- or γ -fluorobutyrate-like toxicity*	Reference
<i>Acids</i>			
Fluoroacetic acid	91c, 92e	Difluoroacetic acid	13
<i>Salts</i>			
Sodium fluoroacetate	13, 20, 34a	Sodium chloroacetate	13, 38c
Sodium γ -fluorobutyrate	13	Sodium bromoacetate	13, 38c
Sodium γ -fluoro- β -hydroxybutyrate	24g	Sodium iodoacetate	13, 38c
Sodium γ -fluorocrotonate	24t	Thallous trifluoroacetate	13
<i>Esters of halogenated acids</i>			
Methyl fluoroacetate	See Table 3	Methyl fluoroformate	13
Ethyl fluoroacetate	13, 91c	Ethyl fluoroformate	91c
Propyl fluoroacetate	91c	Methyl chloroacetate	91c
Isopropyl fluoroacetate	13, 91c	Ethyl dichlorofluoroacetate	13
Allyl fluoroacetate	13		
β -Chloroethyl fluoroacetate	See Table 4		
β -Fluoroethyl fluoroacetate	See Table 4		
β -Chloroethyl fluorothiolacetate	13		
Methyl fluoroselenolacetate	13	Methyl α -fluoropropionate	91f
Methyl γ -fluorobutyrate	See Table 4	Ethyl β -fluoropropionate	20
Ethyl γ -fluorobutyrate	13	Methyl α -fluoroisobutyrate	91c, 92e
Isopropyl γ -fluorobutyrate	13	Ethyl α -fluorobutyrate	13
β -Chloroethyl γ -fluorobutyrate	See Table 4	Ethyl δ -fluorovalerate	91r
β -Fluoroethyl γ -fluorobutyrate	See Table 4	Ethyl ω -fluorohendecanoate	91r
Methyl γ -fluorothiolbutyrate	See Table 4		
Methyl β -chloro- γ -fluorobutyrate	See Table 4		
Methyl γ -fluoro- β -hydroxybutyrate	See Table 4		

TABLE 2 (Continued).

Compounds exhibiting definite fluoroacetate- or γ -fluorobutyrate-like toxicity*	Reference	Compounds exhibiting no or only slight fluoroacetate- or γ -fluorobutyrate-like toxicity*	Reference
Methyl γ -fluoro- β -hydroxythiolbutyrate	See Table 4		
Methyl γ -fluorocrotonate	See Table 4	Diethyl fluoromalonate	91c
Ethyl ϵ -fluorocaproate	91o		
β -Fluoroethyl ϵ -fluorocaproate	91r		
Ethyl ω -fluorocaproate	91r		
	<i>Anhydrides</i>		
Fluoroacetic anhydride	91c, 95e		
	<i>Nitriles</i>		
		Fluoroacetonitrile†	13, 91c
		γ -Fluorobutyronitrile†	13
		γ -Fluorocrotonitrile†	13
		Trifluoroacetonitrile	13
	<i>Aldehydes</i>		
Fluoroacetaldehyde	91o		
	<i>Amides</i>		
Fluoroacetamide	91b, 92b		
N- β -chloroethyl fluoroacetamide	13		
N-nitroso-N-methyl fluoroacetamide	91c		
	<i>Acid halides</i>		
Fluoroacetyl chloride	13, 91c	Acetyl fluoride	13
Fluoroacetyl fluoride	13, 91f	Chloroacetyl fluoride	13, 91c
		Butyryl fluoride	13
		Crotonyl fluoride	13
	<i>Alcohols</i>		
β -Fluoroethanol	See Table 4	γ -Fluoropropanol	59b
	<i>Esters of Fluoroethanol</i>		
	<i>mono(β-Fluoroethyl) derivatives</i>		
β -Fluoroethyl chloroformate	13		
β -Fluoroethyl acetate	91f		
β -Fluoroethyl fluoroacetate	See Table 4		
β -Fluoroethyl chloroacetate	91c, 92g		
β -Fluoroethyl nitrite	13		
Dichloro(β -fluoroethoxy)phosphine	13		
β -Fluoroethyl sulphuryl chloride	91f		
	<i>bis(β-Fluoroethyl) derivatives</i>		
<i>bis</i> (β -Fluoroethyl) carbonate	13	<i>bis</i> (β -Fluoroethyl) fluorophosphate	91f
<i>bis</i> (β -Fluoroethyl) chloromaleate	13		
<i>bis</i> (β -Fluoroethyl) sulfate	13, 91f, 92g		
Di- β -fluoroethyl hydrogen phosphite	91h		
Ethyl <i>bis</i> (β -fluoroethoxy)phosphine	13		
	<i>tris(β-Fluoroethyl) derivatives</i>		
<i>tris</i> (β -Fluoroethyl) orthoformate	13		
<i>tris</i> (β -Fluoroethyl) phosphite	13		
	<i>Ethers and acetals</i>		
β -Fluoroethyl methyl ether	91f	$\alpha, \alpha, \beta, \beta$ -Tetrafluoroethyl β' -hydroxyethyl ether	13
β -Fluoroethyl chloromethyl ether	13		
β -Fluoroethyl β -chloroethyl ether	13		
β -Fluoroethyl phenyl ether	91h		
<i>bis</i> (β -Fluoroethoxy)methane	13		
	<i>Miscellaneous</i>		
<i>bis</i> (β -Fluoroethyl)amine	91p	N-methyl- <i>bis</i> (β -fluoroethyl)amine‡	91p
α -Fluoroacetylsalicylic acid	13, 91h	1-Chloro-2-fluoroethane‡	13, 91f
Fluoroacetyl glycine, ethyl ester	91h, 92f	β -Chloroethyl β -fluoroethyl sulfide	13
Glycol di(monofluoro)acetate	91h, 92f	Trifluoroiodomethane	13
		Fluoroethyl thiocyanate	91h, 92g
		Fluoroethane sulphonyl chloride	91h, 92g
		β -Fluoroethyl triethyl ammonium bromide	91h, 92i
		1,2- <i>bis</i> (β -Fluoroethylthio) ethane	91l

* A compound possessing fluoroacetate-like or γ -fluorobutyrate-like toxicity would, for any species at doses equal to or slightly greater than those listed for methyl fluoroacetate or methyl γ -fluorobutyrate in Tables 3 and 4, produce the characteristic symptoms after the usual latent period (see below), and at least some deaths within 2 days.

† May possess slight activity, but markedly less than corresponding esters.

‡ Produce methyl fluoroacetate symptoms but only at somewhat higher concentrations.

SECRET

TABLE 3. Toxicity of methyl fluoroacetate.

With the exception of the entries marked with an asterisk, the figures are approximations based on limited numbers of observations.

Species	LC_{50} (mg/l) $t = 10$ min	Reference	Intra- venous	Reference	LD_{50} (mg/kg)		Oral	Refer- ence
					Subcutaneous	Reference		
Dog	0.025*	24t	0.08	24t	0.1–0.2	73	0.1–0.2	73
Cat	(0.025–0.05)†	24i	0.2*	40b	0.3	73	0.3	73
Rabbit	0.065*	24t	0.33*	24t	0.3–0.5	24i, 73	0.5	73
Guinea pig	0.15	24d, 91c	...	...	0.2	73	0.5	73
Goat	0.2	76	<2.0	51	1.0	73	1.0	73
Rat	0.3	24d, 73, 76	...	...	2.5	73	3.5	73
Mouse	3.2*	24f,t	17*	24t	5–20	24i, 73	(5–6)	24j, 73
Rhesus monkey	0.8–2.0	73, 76	5–15	51	10–12	73	10–12	73
Cercopithecus monkey		...	...	...	>50‡	91q	...	...
Frog		...	...	...	100–200	40a	...	...

† Estimate based on susceptibility to β -fluoroethanol.

‡ Intraperitoneal injection.

characteristic toxicological properties of methyl fluoroacetate and methyl γ -fluorobutyrate. Compounds which produce the characteristic toxicological actions fall into the following categories:

1. The following acids, in some cases tested only as salts and esters: fluoroacetic, γ -fluorobutyric, γ -fluoro- β -hydroxybutyric, β -chloro- γ -fluorobutyric, γ -fluorocrotonic, ϵ -fluorocaproic, and ω -fluorocapric.

2. Other simple derivatives of the above acids and their thiol analogs, including anhydrides, amides, aldehydes, and acid halides, but not the nitriles.

3. β -Fluoroethanol, its esters, and certain other derivatives.

The following compounds do not evoke the characteristic toxic effects:

1. Di- and poly-fluoro derivatives of the toxic mono-fluoro compounds.

2. Chlorine, bromine, and iodine analogs of the toxic fluorinated derivatives.

3. Fluoride-liberating compounds such as acid fluorides.

4. Derivatives of aliphatic acids in which the fluorine atom is not in the terminal position (i.e., methyl α -fluoropropionate, methyl α -fluoroisobutyrate, ethyl α -fluorobutyrate, and diethyl fluoromalonate).

5. ω -Fluoro derivatives of aliphatic acids with an odd number of carbon atoms (e.g., ethyl β -fluoropropionate, ethyl δ -fluorovalerate, and ethyl ω -fluorohendecanoate).

Thus, the $F\cdot CH_2$ -group appears to be essential. Its presence is not sufficient, however, and presumably it must form the end of a chain of an even number of carbon atoms. It is also necessary that the proper group, usually an oxygenated one, form the

other end of the chain (e.g., methyl fluoroacetate is highly toxic, 1-chloro-2-fluoroethane is less so, and fluoroacetonitrile is relatively nontoxic). That other features of the molecules play a role in determining the degree of toxicity by inhalation is also revealed by the large differences which exist between the precisely determined LC_{50} 's for a number of related derivatives containing one and two $F\cdot CH_2$ -groups,^{24i,s,44} and by the large differences in toxicity which are associated with various β -substitutions in methyl γ -fluorobutyrate (see Table 4).

10.4

TOXICOLOGY

10.4.1

Toxicity for Animals

The toxicity of methyl fluoroacetate for animals is set forth in Table 3 and may be evaluated in comparison with hydrogen cyanide, the LD_{50} of which is in the order of 1 mg/kg for most species, including man (see Chapter 2). It is noteworthy that: (1) the species variation is unusually large — the dog is approximately 100 times more susceptible than the mouse or monkey and two tested species of monkeys show considerably different susceptibilities; and (2) the compound is approximately as toxic when administered by mouth as when injected intravenously or subcutaneously.

The toxicity of β -fluoroethanol for various species is comparable to that of methyl fluoroacetate; β -fluoroethyl fluoroacetate, the most toxic member of the fluoroacetate group, is somewhat more potent (Table 4). Methyl γ -fluorobutyrate and related compounds (Tables 2 and 4) produce toxic effects similar in a general way to those of methyl fluoroacetate but exhibit less pronounced species variation, principally

TABLE 4. Inhalation toxicities of fluorinated aliphatic compounds.
With the exception of the mouse LC_{50} 's, the figures are approximations based on limited data.

Compound	LC_{50} (mg/l, nominal, for $t = 10$ min)						
	Monkey (Rhesus)	Mouse	Rat	Guinea pig	Rabbit	Cat	Dog
Methyl fluoroacetate	0.8–2.0 ^{73,76}	3.2 ^{24f,t}	0.3 ^{24d,73,76}	0.15 ^{24d,91c}	0.065 ^{24t}		0.025 ^{24t}
β -Chloroethyl fluoroacetate		0.7 $\pm$ 24d,91c	0.2 $\pm$ 91c	0.15 $\pm$ 24d,91c	0.1 ^{91c}		
β -Fluoroethyl fluoroacetate		0.63 ^{24g}	0.2 $\pm$ 91d	0.07 ^{24d,91d}	0.05 ^{91d}		
β -Fluoroethanol	1.5 ^{24d,e}	1.2 ²⁴ⁱ	0.2 $\pm$ 24d,76	0.15 ^{24d,91c}	0.025 ^{24d}	0.035 ^{24d}	(0.007) ^{24d}
Methyl γ -fluorobutyrate	0.5 ^{24h}	0.12 ²⁴ⁱ	0.35 $\pm$ 24h	0.07 ^{24h}	0.035 ^{24h}	0.035 ^{24h}	0.05 ^{24h}
Methyl γ -fluorothiolbutyrate		0.064 ²⁴ⁱ					
β -Chloroethyl γ -fluorobutyrate	>0.3 ^{24j}	0.054 ^{24j}		0.1 $\pm$ 24j			
β -Fluoroethyl γ -fluorobutyrate	0.5 $\pm$ 24h	0.077 ²⁴ⁱ	0.2 ^{24h}	0.035 ^{24h}	<0.075 ^{24h}	0.025 ^{24h}	0.025 ^{24h}
Methyl β -chloro- γ -fluorobutyrate		0.16 ²⁴ⁱ					
Methyl γ -fluoro- β -hydroxybutyrate	0.2 ^{24p}	0.023 ^{24m}			<0.063 ^{24p}	0.1 ^{24p}	<0.063 ^{24p}
Methyl γ -fluoro- β -methoxybutyrate		>0.1 ^{59b}		>0.1 ^{59b}			
Methyl γ -fluoro- β -hydroxythiolbutyrate	0.2 ^{24p}	<0.03 ^{24p}			<0.063 ^{24p}		0.063 ^{24p}
β -Chloroethyl γ -fluoro- β -hydroxybutyrate		0.048 ^{24s}					
Methyl γ -fluorocrotonate	<0.5 ^{24h}	0.089 ²⁴ⁱ		0.15 ^{24h}			

because of a much greater toxicity for mice. When tested on monkeys, the members of this group are more toxic than methyl fluoroacetate but not so toxic as either mustard gas or phosgene.

Methyl fluoroacetate, and presumably also the γ -fluorobutyrate derivatives, are detoxified in the body, but only at a slow rate.^{24d,g,i,40a,73,76,91f,q} Changes in $L(Ct)_{50}$ with changes in exposure time over the range 1 to 100 minutes have been observed in experiments with methyl fluoroacetate, β -fluoroethyl fluoroacetate, and β -fluoroethanol, but the effects are not large.^{24d,g,73,76} The $L(Ct)_{50}$ of methyl γ -fluorobutyrate for mice is the same for exposures of 1, 10, and 100 minutes,^{24g} and that of methyl γ -fluorocrotonate may not be significantly different for exposures of 10 or 100 minutes or for two fractional exposures at a 24-hour interval.²⁴ⁱ Summation of the effects of multiple sublethal doses of methyl fluoroacetate administered at daily intervals by mouth, injection, or gassing has been observed but some detoxification occurs and, with sufficiently small increments, the equivalent of several lethal doses can be tolerated.^{40a,73,91f} However, species differences appear to exist; successive small doses produce a more pronounced cumulative effect in guinea pigs than rats, the latter species probably developing an increased resistance to the poison.^{91f} Indeed, recent data dem-

onstrate that a small dose (approximately 0.1 LD_{50}) administered orally or subcutaneously confers a statistically significant degree of resistance upon rats tested 24 hours later with an LD_{50} administered orally or intramuscularly.^{57c} A similar phenomenon has been reported for orally administered sodium fluoroacetate, but it would not appear that the elevation of resistance is sufficient to affect the value of the salt as a rodenticide.

A characteristic latency is associated with the visible effects of poisoning by methyl fluoroacetate and related compounds. Even 10 to 20 times the lethal dose produces symptoms only after a minimum delay of 15 minutes.^{24d} Survival times of animals dying as a result of inhalation of median lethal dosages are almost always at least 1 hour, usually 2 to 12 hours, less frequently 12 to 24 hours, and rarely longer.^{24d,91c} The derivatives of γ -fluorobutyric acid act similarly to the fluoroacetates but the latent period may be somewhat briefer and the recovery of sublethally poisoned animals more protracted.^{24g,h,j}

There are two immediate causes of death in methyl fluoroacetate poisoning: action on the heart, culminating in ventricular fibrillation and circulatory failure; and stimulation of the central nervous system, producing convulsions, apnea, and death with-

out severe cardiac^c abnormalities.⁵¹ The relative severity of the two effects is not the same in different species: the cardiac action is the primary cause of death in monkeys, goats, and rabbits; effects on the central nervous system predominate in rats, cats, and dogs.^{40,51,58k,73} Transient but sublethal central nervous effects occur in some species (e.g., *Rhesus*) which eventually die with ventricular fibrillation, and cardiac effects in other species (e.g., the cat) which die of respiratory failure following severe convulsions.⁵¹ The poisoned heart has a decreased excitability and the effects are not due to diminished coronary blood flow.⁵¹

In experiments on three monkeys methyl γ -fluorobutyrate produced cardiac depression and arrhythmias, as well as marked parasympathetic symptoms, but ventricular fibrillation has not been observed.^{58h} β -Fluoroethyl γ -fluorobutyrate, likewise tested on only three monkeys, produced effects similar to those of methyl fluoroacetate but was more toxic.^{58h} Both compounds produced effects similar to methyl fluoroacetate in the cat and rabbit.^{58f}

The symptoms associated with poisoning by methyl fluoroacetate and related compounds have been described in detail for various species^{24d,g,h,j,q,51,58k,73,91a} and can be interpreted as resulting from the actions of the poisons on the heart or nervous system, or both.

Pathological studies^{24a,d,h,73,91k,q} in animals dying acutely from single doses reveal no significant changes other than signs referable to venous congestion. In animals exposed repeatedly to sublethal doses until death ensues,^{91k,q} there are found the sequelae of protracted venous congestion attributable to heart failure, definite abnormalities in the myocardium, changes in the kidney which may or may not be secondary to disturbances in the metabolism of other organs, and changes of doubtful significance in some other organs; no unequivocal pathological changes have been observed in the nervous system.

10.4.2 Physiological Mechanism

A number of clinical pathological and biochemical studies have been made to throw light on the cellular mechanism of action of methyl fluoroacetate and related compounds.^{18,24d,f,28,34,38,40,51,58b,f,j,k,l,73,91a,b,g,j,}

^c It may be noted that the conclusions concerning cardiac effects have been based on detailed, continuous electrocardiographic observations,⁵¹ and that species which exhibit central nervous stimulation concomitantly develop abnormal electroencephalograms in the absence of notable cardiac irregularities.^{57b}

^{m,n,q,93,98} Their heterogeneous character precludes a review of all the isolated facts which eventually may prove to be of significance.

The evidence is strong that methyl fluoroacetate does not owe its toxicity to the liberation of fluoride ion at critical loci in the body. In accord with chemical studies on the stability of the fluorine atom (see Section 10.2.3), none of a large number of biochemically important substances,^d including some with a high reactivity toward organic halogens, liberates fluoride from methyl fluoroacetate at physiological conditions of pH and temperature;^{28,38a} nor is fluoride ion liberated when the ester is incubated with rat tissues.¹⁸ Moreover, methyl fluoroacetate does not show a marked tendency to inactivate enzymes which are highly susceptible to fluoride.^{38a}

It has been proposed as a working hypothesis that all the toxicologically active compounds under consideration may be the precursors of some common toxic material, possibly the fluoroacetate ion, which could be produced, for example, by hydrolysis of esters, oxidation of β -fluoroethanol, and β -oxidation of the γ -fluorobutyrate.⁹¹ⁱ Although this hypothesis, which conceivably could explain the facts set forth in Section 10.3, "Chemical Structure in Relation to Toxicity," has not as yet been submitted to systematic test, the following findings may be cited as bearing upon it.

1. Sodium fluoroacetate, fluoroacetic acid, and fluoroacetamide possess approximately the same toxicity as methyl fluoroacetate and produce symptoms after a comparable latency.^{51,91b} The latency in poisoning by the ester is not, therefore, determined by time for hydrolysis. However, this does not imply that hydrolysis of the ester may not be a necessary prelude to the initiation of toxic action. Tissues and blood contain a methyl fluoroacetate esterase,^{18,38a} which in the rat is sufficiently active to afford the ester a half life of not more than a few minutes — a fraction of the usual latent period for symptoms.¹⁸

2. That the characteristic effects of methyl fluoroacetate and sodium fluoroacetate on the myocardium do not require *in vivo* chemical changes in *other* organs is suggested by experiments on eviscerated rabbits⁵¹

^d Arginine, serine, histidine, tyrosine, proline, asparagine, glutamic acid, lysine, tryptophane, alanine, glycyl glycine, imidazole, guanidine, cysteine, glutathione, S-allyl thiourea, β -mercaptoethanol, 2,3-dimercaptopropanol, carbobenzoxy methionine, thiodiglycol, benzylamine, triethanolamine, tetraethanolammonium chloride, hexamethylenetetramine, and β -aminobenzoic acid; or sodium thiosulfate, sodium sulfide, sodium bisulfite, or sodium iodide.

and proved by tests with isolated, perfused hearts of the cat,⁵¹ rabbit,^{91m} and guinea pig,⁹¹ⁿ and with the isolated papillary muscle of the cat.⁵¹ A similar conclusion with respect to effects on the central nervous system is suggested by the finding that local application of methyl fluoroacetate to one cerebral hemisphere produced convulsive discharges after the usual latency for symptoms; although the convulsions were generalized, the effect of the poison on the treated hemisphere appeared to be greater than on the contralateral areas.^{91g}

3. On the contrary, it may be necessary for β -fluoroethanol to undergo chemical change, possibly by oxidation to fluoroacetate in the liver. This is suggested by the finding that the alcohol exerted no effect on the isolated, perfused heart when tested at concentrations at which methyl fluoroacetate produced marked decreases in rate and survival time.^{91m}

4. If the toxicity of the γ -fluorobutyrate and other longer-chained fluorinated aliphatic acids depends on the production of fluoroacetate by β -oxidation, their relatively high toxicity for some species (Table 2) would require that they be concentrated to a greater degree than the fluoroacetates at critical loci in the body. That the β -oxidation of γ -fluorobutyrate is not prerequisite for *all* its actions upon biological systems is indicated by evidence that methyl γ -fluorobutyrate is not converted to fluoroacetate by rabbit kidney cortex *in vitro*, in spite of the fact that both compounds markedly inhibit the oxidation of acetate by this preparation.^{38a} Substitutions on the β -carbon atom are, however, important determinants of inhalation toxicity, as is revealed by the widely differing toxicities of a number of the butyric acid derivatives listed in Table 4.

Changes indicative of a derangement in carbohydrate metabolism in methyl fluoroacetate poisoning in various mammalian species are increases in blood sugar,^{58b,73} nonprotein nitrogen,^{40a,73} inorganic phosphate,^{58j} lactic acid,^{40a,58b,j} pyruvic acid,^{58b} and lactate-pyruvate ratio.^{58b} In rabbits there is a marked reduction in liver glycogen^{58j} and, in the heart, marked decreases in total acid-soluble phosphorus and organic soluble phosphorus.^{58j} Serum potassium and calcium show only minor increases.^{58k,73}

Negative results have been obtained in many but not all the studies on the effects of fluoroacetate on enzyme systems *in vitro* and on the metabolism of tissues obtained from poisoned animals or treated with the poison after isolation.^{18,34,38,40,58b,f,l,91a,98}

Illuminating experiments have been performed

with an isolated skeletal muscle, the sartorius of the frog, but have not been extended as yet to cardiac muscle.^{18,40b,c} The resting oxygen consumption and the contractility of the unfatigued sartorius are not affected by the poison at a concentration of 0.005M, but the extra oxygen consumption following activity is strongly inhibited.^{18,40b,c} The inhibition is associated with a greatly decreased resynthesis of phosphocreatine¹⁸ and abolition of the delayed heat production normally associated with aerobic recovery of stimulated muscle.^{40b,c} Similarly, the extra oxygen consumption produced by pretreatment of the isolated muscle with the stimulants caffeine and dinitrophenol is essentially abolished by fluoroacetate.^{18,40c} Similar changes are produced by sodium azide but the mechanism of action is different: azide inhibits cytochrome oxidase and adenylyl pyrophosphatase, whereas methyl fluoroacetate has no inhibitory action either upon these enzymes or upon cytochrome reductase.¹⁸

The possibility that fluoroacetate inhibits lactic acid dehydrogenase is suggested by the findings that the isolated frog sartorius utilizes pyruvate (also acetate) but does not oxidize added lactate,^{40c} and that the effects of fluoroacetate upon the isolated guinea pig heart are counteracted by pyruvic acid derivatives but not by sodium lactate.^{91m} An *in vitro* study on lactic acid dehydrogenase likewise revealed an inhibition of the enzyme prepared from yeast,^{40d} although in another experiment the enzyme prepared from heart muscle was not reported to be inhibited by methyl fluoroacetate.^{38c} Data relating to the production of lactate by the stimulated poisoned muscle under anaerobic conditions are not consistent.^{18,40c} In the case of rabbit kidney cortex preparations *in vitro*, however, methyl fluoroacetate inhibits the oxidation of glucose and certain intermediates of carbohydrate metabolism but it has no effect on the anaerobic phase of carbohydrate degradation resulting in the formation of lactic acid. The latter findings have led to the suggestion that a locus of action may be at one of the steps in the dehydrogenation of pyruvate via the citric acid cycle.¹⁸

The effect of fluoroacetate on the oxygen consumption of stimulated skeletal muscle has been found to be reversible,¹⁸ offering some hope that methyl fluoroacetate poisoning may eventually be subject to treatment. Moreover, if the oxygen consumption of heart muscle should prove to be as easily inhibited by methyl fluoroacetate as that of stimulated skeletal muscle, the possibility would

exist that a therapeutic agent could be found in a carbohydrate intermediate the oxidation of which is not strongly inhibited.¹⁸

The slight therapeutic value of procaine and *p*-aminobenzoic acid in fluoroacetate-poisoned monkeys and the absence of a corresponding effect with other antifibrillatory drugs (see below) suggested the alternative possibilities that *p*-aminobenzoic acid might be fluoroacetylated, thereby detoxifying the poison, or that the toxicity of the fluoroacetate might be associated with an inhibitory action on normally occurring acetylations. However, experiments reveal that the monkey does not acetylate, and therefore probably does not fluoroacetylate, *p*-aminobenzoic acid,⁵⁸ⁱ that the acetylation of *p*-aminohippuric acid by rabbits is not markedly affected in fluoroacetate poisoning,^{34b} and that fluoroacetate does not inhibit the acetylation by liver slices of sulfanilamide, *p*-aminobenzoic acid, or choline.^{38a,b} On the other hand, fluoroacetate does produce some inhibition of the utilization of acetate *in vitro* by rabbit heart and kidney¹⁸ preparations and by rat kidney, liver, and heart slices.^{38b} In the case of *Corynebacterium creatinovorax* and of yeast, the inhibition is almost complete.^{38a} These and other findings have led to the suggestion that fluoroacetate may produce a profound alteration in the metabolism of carbohydrate by virtue of a specific inhibitory effect on the oxidation of acetate.^{38c} However, poisoned caffeine-stimulated muscle does utilize acetate.^{40c}

The resting potential of frog peripheral nerve is sensitive to concentrations of methyl fluoroacetate as low as 0.001*M*.^{34c,d} The potential was reduced in poisoned nerves by a period of anoxia, and the oxidative recovery was little affected by addition of acetate or acetyl phosphate, but was counteracted by addition of pyruvate.

10.4.3

Therapy

No satisfactory procedures for the treatment of fluoroacetate poisoning have been discovered. Tests have been made of substances and procedures designed to prevent convulsions, to stimulate respiration, to stimulate diuresis and excretion of the poison, to prevent ventricular fibrillation and otherwise restore the failing heart, to promote detoxification by fluoroacetylation, to compete for enzyme systems with fluoroacetate, and to supply necessary metabolites the formation of which may be cut off by the action of the poison on enzyme systems.^{18,24e,f,51,58c,e,i,j,k,l,73,91g,j,m}

Intracardiac injections of procaine accompanied by artificial respiration and cardiac massage through the thoracic wall temporarily restore an organized beat to the monkey heart fibrillating as a result of methyl fluoroacetate poisoning; but fibrillation recurs and eventually proves fatal in spite of continued treatments and the presence of subcutaneous deposits of procaine.^{51,58d}

Administration of large doses of sodium *p*-aminobenzoate to anesthetized monkeys (*Rhesus* and *Ateles*) poisoned with one *LD*₁₀₀ dose of methyl fluoroacetate corrects the cardiac disturbances and saves the majority of animals.^{58i,j} However, the value of this treatment is limited inasmuch as it does not save monkeys poisoned with larger doses^{58j} or rabbits poisoned with four *LD*₅₀'s;^{91m} nor does it combat the lethal action of methyl fluoroacetate in the rat, a species which dies of central nervous rather than cardiac effects.^{58k}

Large concentrations of sodium acetate (0.1 per cent) prolong the survival of the isolated rabbit heart perfused with depressant concentrations of methyl fluoroacetate, but the acetate ion has exerted little or no protection when administered to the poisoned animal.^{58l} Similarly, the sodium salt and other derivatives of pyruvic acid protect the isolated guinea pig heart poisoned with methyl fluoroacetate but have little or no therapeutic value *in vivo*.^{91m}

Various anesthetics have been shown to be effective in controlling the convulsions associated with methyl fluoroacetate poisoning,^{24e,51,91g,j} but even when combined with respiratory stimulants they do not decrease the mortality.^{91g} Sodium pentobarbital is contraindicated because it increases the mortality.^{91j}

The following additional substances and procedures have been without significant value *under the tested conditions* in saving the lives of animals poisoned with methyl fluoroacetate or β -fluoroethanol: artificial respiration;^{91g} artificial respiration plus sodium phenobarbital;^{24f,73} oxygen plus carbon dioxide;⁷³ urethane, paraldehyde, or chloral hydrate, with or without theophylline or coramine;^{91g} theophylline;^{58e,91j} bromide;^{91g} Dilantin (sodium diphenyl hydantoin);^{73,91g} morphine hydrochloride;^{91g,j} quinidine, digitalis, quinidine-digitalis, or caffeine;^{58e} papaverine;^{58j} yohimbine;^{58l} anticholinesterase drugs or aconite;^{58e} atropine;^{24e,58e} ephedrine;^{58c,e} thiamine;^{58e} glucose;^{24e} potassium salts;^{58e,91m} calcium salts;^{24e,58e} barium salts;^{58e} 2,3-dimercaptopropanol (BAL), acetophenone, or cobalt acetate.^{91m}

10.4.4 Toxicity for Man

Man is among the species which are relatively resistant to methyl fluoroacetate. Direct evidence comes from the results of ingestion of the compound by a British volunteer.^{91j} Upon taking an oral dose of 0.4 mg/kg in water he experienced no symptoms other than a slight, possibly psychogenic, feeling of unsteadiness upon standing up 1½ hours after the dose was taken. Similar ingestion of 0.65 mg/kg produced no symptoms other than a feeling of unsteadiness for a few minutes 1 hour after the dose and a slight malaise 5 hours later; however, the subject continued work in the laboratory with no obvious loss of efficiency and his electrocardiogram and electroencephalogram, recorded at frequent intervals, showed no deviation from the normal. It is to be noted that the dose ingested was greater than the LD_{50} for guinea pigs, rabbits, cats, and dogs.

Various lines of evidence^{73,80} suggested that the lethal dose *per os* is in the order of 6–8 mg/kg. Exposure of workers for prolonged periods to low concentrations of the vapor⁹⁶ produced marked weakness, reluctance for any physical effort, and strong mental depression with periods of nervous irritation difficult to control, followed by physical and mental exhaustion, drowsiness, and giddiness; a few days' rest resulted in marked improvement.^e

Assuming (1) that the above estimate of the lethal dose *per os* for man is correct, (2) that the toxicity of methyl fluoroacetate is more or less independent of the route or rate of administration, and (3) that 100 per cent absorption of inhaled vapor occurs, one may calculate that the lethal vapor dosage for a 70-kg man breathing 10 lpm (relative inactivity) would be 50,000 mg min/m³, corresponding to a 10-minute LC_{50} of 5 mg/l; for a man breathing 40 lpm, corresponding to exercise intermediate between a walk at 5 mph and a slow run,² the figure would be 12,500 mg min/m³, the equivalent of 1.25 mg/l for 10 minutes. Although the validity of this method of calculation has been questioned,⁷⁶ it has been shown to yield good approximations when applied to the dog and rabbit, the only larger species for which both the LC_{50} 's and LD_{50} 's have been determined with precision.^{24t} For defensive purposes the British have estimated the $L(Ct)_{50}$ at 4,000 and 7,000 mg min/m³.^{76,79} For most species the margin between the convulsive and lethal doses is small.

^e These symptoms, experienced by Polish chemists, prompted the initial toxicological examination of the effects of methyl fluoroacetate on animals.

The mild, indistinctive odors of methyl fluoroacetate and β -fluoroethanol make it possible that large vapor dosages could be inhaled undetected. It has been reported that methyl fluoroacetate at 0.05 mg/l is just detectable, that at 0.2–0.3 mg/l it would easily be overlooked, and that at 0.4 mg/l it possesses a fruity smell and may produce a slight feeling of tightness in the chest.^{79,91c} Most of the γ -fluorobutyrate is probably somewhat more odorous than methyl fluoroacetate but it has been emphasized that methyl γ -fluoro- β -hydroxybutyrate possesses only a very slight odor, similar to but much fainter than that of ethyl lactate.^{24p}

A prominent symptom in severe poisoning is the occurrence of repeated and severe convulsions indistinguishable from status epilepticus;⁷³ less dramatic symptoms may include nausea, vomiting, dizziness, and fall in body temperature.⁹¹ⁿ In addition to the symptoms, tests with urine may aid in the recognition of fluoroacetate poisoning, for a toxic, fluorine-containing substance not present in normal urine is excreted.^{91g,j,n,q} The fluorine may be converted to fluoride and detected by chemical test⁹¹ⁿ or the urine given to rats by stomach tube, the characteristic symptoms of fluoroacetate poisoning⁷³ then being produced.^{91n,q}

In the absence of specific therapeutic procedures for fluoroacetate poisoning, cases can at present only be treated symptomatically. Morphine has been recommended to allay distress, anxiety, and convulsions, but barbiturates (i.e., pentobarbital sodium) are contraindicated.^{91g}

10.5 EVALUATION AS WAR GASES

Evaluation of the potentialities of methyl fluoroacetate and related compounds in terms of available data and present concepts of chemical warfare indicates that none of the derivatives possesses the general utility of currently standardized gases. They remain a subject of some military concern, however, in view of their potential use as food and water poisons (see Section 10.6) or for other special purposes.

For man methyl fluoroacetate is not appreciably more toxic, and in all probability is considerably less toxic, than currently standardized gases. The lethal vapor dosage, calculated above on the basis of the demonstrated low oral toxicity to be in the order of 12,000 mg min/m³ for ventilation rates corresponding to moderate physical activity and several times

this value for men at rest, may be compared with the minimum dosages of standard agents currently recommended as adequate for the following tasks:⁷¹

Task	Agent	Dosage (mg min/m ³)
To produce a large proportion of deaths or severe casualties in surprise attacks with nonpersistent gases (dosages to be obtained within 2 minutes)	Phosgene	3,200
	Hydrogen cyanide	5,000
	Cyanogen chloride	11,000
To produce skin burns of sufficient severity to totally disable 50 per cent of <i>masked</i> troops not equipped with protective clothing (dosages to be obtained within 4 hours)	Mustard vapor	1,000 ($T > 80\text{ F}$)
		2,000–4,000 ($T = 60\text{--}80\text{ F}$)
To produce eye damage of sufficient severity to cause temporary blindness among troops not wearing gas masks	Mustard vapor	200

In view of the relatively low toxicity for man, it is apparent from a consideration of the physical properties of methyl fluoroacetate (Table 5), the most volatile stable compound of the group,^f that it would

TABLE 5. Physical properties of methyl fluoroacetate and of currently standardized nonpersistent agents.

Property	Methyl fluoro- acetate	Hydro- gen cyanide	Cyano- gen chloride	Phosgene
Liquid density (g/ml at 25 C)	1.17	0.68	1.2	1.36
Boiling point, C	104	26	12.6	8.3
Freezing point, C	–35	–13.4	–7	–104
Latent heat of evap- oration, cal/g	100	210	135	60
Vapor pressure, mm Hg				
at 25 C	20	740	1,200	1,400
at –20 C	...	88	180	230
Volatility, mg/l				
at 25 C	119	1,060	...	...
at –20 C	...	145	680	1,460

be more difficult than in the case of the standard nonpersistent gases to achieve in the field vapor dosages sufficiently large to be lethal in surprise attacks; and, in view of the effectiveness of the canister,⁸⁰ the breaking of the gas mask cannot be considered a feasible task.

The volatilities of β -fluoroethanol and of various stable fluoroacetate derivatives having toxicities

^f The more volatile β -fluoroethyl nitrite, fluoroacetyl fluoride, and fluoroacetyl chloride are chemically unstable.

comparable to methyl fluoroacetate range from that of methyl fluoroacetate (i.e., 119 mg/l at 25 C) down to very low values. Thus, agents of any desired degree of persistence are potentially available. Although the indistinctive odor and relative difficulty of detection by chemical means would confer upon these compounds a certain insidiousness, their lack of effectiveness on the eyes and skin renders them inferior in general utility as persistent agents to such vesicants as mustard gas and *tris*(β -chloroethyl)-amine (HN3). Except in drinking water, their decontamination offers no special problems.⁸⁰

Methyl fluoroacetate possesses excellent storage stability (see Section 10.2.2) and its explosion stability is believed to be sufficient to permit its dispersal from chemical munitions now in use.^{24j} It is potentially available in quantity.

There are no data bearing upon the toxicity for man of the derivatives of γ -fluorobutyric acid. On the basis of the comparative toxicities of these derivatives and of methyl fluoroacetate for the monkey (Table 4), they would be suspected of being somewhat more toxic for man than is methyl fluoroacetate. They are, however, less volatile (Table 1) and notably more difficult to manufacture.

10.6 POTENTIAL EFFECTIVENESS AS FOOD AND WATER POISONS IN WARFARE^{21,24c,e,73,79,85}

The chemical and toxicological properties of methyl fluoroacetate and related compounds make them potential water and food poisons. They are approximately as toxic when administered orally as when injected or inhaled; to a degree they may act as cumulative poisons; and they are not readily detected by smell or taste. Although methyl fluoroacetate itself undergoes hydrolysis, the resulting fluoroacetic acid is stable and toxic.

At concentrations of 0.1 per cent or less in water, methyl fluoroacetate has no smell or taste;⁸⁵ $\frac{1}{2}$ l of a 0.1 per cent solution would probably be lethal for man (see Section 10.4.4). This concentration in milk is readily accepted by rats and dogs,⁸² although it is not freely accepted in otherwise pure drinking water by rats, nor are β -fluoroethanol and sodium fluoroacetate at 0.01 per cent (100 parts per million) freely accepted by mice.^{24c,e} The effectiveness of sodium fluoroacetate as a rodent bait poison is discussed below.

Filtration of contaminated water with charcoal

removes methyl fluoroacetate but not the hydrolytic product, fluoroacetic acid.⁸⁵ Filtration with charcoal plus pyridine is said to remove not only methyl fluoroacetate but also, from neutral solution, sodium fluoroacetate as well.⁴⁴ The detection of fluorine compounds in contaminated water is discussed in Chapter 34.

10.7 USE AS RODENTICIDES

Sodium fluoroacetate was one of several substances studied in the chemical warfare program in the United Kingdom and the United States which were recommended by Division 9 of NDRC to the Fish and Wildlife Service of the Department of Interior for test as rodenticides.⁴² Preliminary tests with small samples (200 lb) submitted by Division 9 were so successful that the division subsequently prepared an additional 1,000 lb²² for large-scale field trials. Field campaigns in a number of states and in military establishments in this country and abroad were conducted by the Fish and Wildlife Service, by the Typhus Control Unit of the Public Health Service, and by the medical departments of the Army and Navy. The results demonstrate that sodium fluoroacetate, coded 1080, is one of the most promising available rodenticides.^{45d,60-70}

Sodium fluoroacetate possesses the following requirements of a good rodenticide: high toxicity and acceptability, stability, lack of volatility, lack of irritation and toxic properties for human skin, lack of inflammability, and potential availability in quantity at reasonable cost.^{45b} The oral lethal doses for

various species of rats and other rodents of concern in public health and agriculture range between 0.1 and 5 mg/kg.^{41a,100} The substance is effective in baits at much lower concentrations than in the case of other rodenticides. Excellent results have been obtained in field trials utilizing 6 oz of sodium fluoroacetate per 250 lb of cereal or ground meat bait.^{41b} Water solutions are also highly effective. A concentration of 1/2670 has been recommended for general use,⁷⁰ although concentrations ten times greater (i.e., 5 oz gal) are sufficiently acceptable to rats and have been used with good results.^{41b}

As in the case of other rodenticides, the possibility of accidental human poisoning cannot be ignored, particularly in the absence of effective methods for treatment of fluoroacetate poisoning. However, it would appear that concentrations sufficiently low to make accidental human poisoning improbable may still be effective in rodent control. The human lethal dose is believed to be of the order of 5-10 mg/kg (see Section 10.4.4). On the other hand, the oral lethal doses for cats and dogs are very low (0.1 to 0.5 mg/kg) and, therefore, the likelihood of accidental poisoning of these species confers a certain disadvantage upon sodium fluoroacetate.

The sodium salts of γ -fluorobutyric, γ -fluoro- β -hydroxybutyric, and γ -fluorocrotonic acids are several times as toxic for rats as is sodium fluoroacetate. However, in view of the already high toxicity of the latter, this apparent disadvantage is more than offset by the greater difficulty and expense of their preparation.

Chapter 11

CADMIUM, SELENIUM, AND THE CARBONYLS OF IRON AND NICKEL

By John A. Zapp

11.1 INTRODUCTION

IN THE SEARCH for new chemical warfare agents, the toxic properties of certain metals were not neglected. The increasing use of cadmium in industry, for example, had revealed that the inhalation of finely divided cadmium metal, the oxide, or salts was capable of producing severe lung edema comparable with that produced by phosgene.⁶⁶⁻⁶⁸ Selenium compounds showed similar properties,⁶⁹ and, although somewhat less toxic than cadmium on an absolute basis, they produced physiological effects much more promptly. Being inorganic, these agents offered promise for inclusion in burning-type munitions or incendiaries as well as for dispersion by high-explosive shell. The carbonyls of iron and nickel aroused considerable interest not only because of their inherent toxicity, but also because they break down catalytically in contact with gas mask carbon, yielding carbon monoxide which is not absorbed in the canister but passes into the mask.^{9a,b} Thus the carbonyls might be valuable in attacking either masked or unmasked troops. Compounds of mercury, thallium, tin, antimony, lead, chromium, and germanium were screened by the University of Chicago Toxicity Laboratory [UCTL],⁷ but without revealing any of special interest for chemical warfare purposes.

The part of Division 9 of the National Defense Research Committee [NDRC] in the field of heavy metals was largely one of screening the toxicity of a great number of compounds, many of which were prepared under Office of Scientific Research and Development [OSRD] contracts. The detailed investigation of the promising compounds, including investigations of cadmium and selenium and the carbonyls, was carried out by the Chemical Warfare Service and by the Directorate of Chemical Warfare in Canada.

11.2 CADMIUM

11.2.1 Physiological Action

Cadmium, its oxide, and salts are toxic by any route of administration, but their particular signifi-

cance in chemical warfare lies in the fact that finely divided dusts can be set up either by thermal combustion of incendiary mixtures containing cadmium or by the explosive dispersal of cadmium compounds. These dusts are quite toxic by inhalation,⁶⁶⁻⁶⁸ producing lung edema comparable with that observed in phosgene poisoning.

Exposure to high concentrations of cadmium causes some early respiratory irritation, which progresses to marked dyspnea within a few hours. Two cats exposed to a high concentration of cadmium oxide fume for 30 minutes⁶⁶ showed on autopsy extensive acute pulmonary injury with edema, injury to the bronchioles and alveolar ducts, and acute alveolar emphysema. Liver and kidney damage was also found. Exposure to lower concentrations of cadmium fumes or dust results in a temporary irritation of the respiratory tract which disappears shortly after cessation of exposure only to reappear within about 12 hours with increasing severity accompanied by general malaise. Within about 24 to 48 hours dyspnea is marked and cyanosis occurs prior to death.^{10,17,18,66} On autopsy the lungs are found to be firm, but with interstitial and perivascular edema and extensive hemorrhage. Liver and kidney show evidence of fatty infiltration.

Several cases of human poisoning by inhalation of cadmium have been reported. In one of these, reported in 1858,⁶⁶ three men were exposed to cadmium carbonate dust. Symptoms did not occur until several hours after exposure and then consisted of dyspnea, dizziness, vomiting, and diarrhea. One patient apparently contracted pneumonia by secondary infection, but all three recovered eventually. Fifteen cases of human cadmium poisoning from inhalation of cadmium oxide fumes, two of which were fatal, have been reported from Canada.⁶⁷ In all these cases, dyspnea, which did not become severe until several hours after exposure, was the most prominent symptom, although the majority of cases also exhibited gastrointestinal symptoms. The two men who died showed congestion of the lungs, pulmonary edema, hemorrhage into the lungs, atelectatic areas, proliferative interstitial pneumonitis, and catarrhal

bronchitis. Liver and kidney damage was also present.

11.2.2 Toxicology

The toxicity of cadmium oxide by inhalation is summarized in Table 1 for various species and ex-

TABLE 1. Toxicity of cadmium oxide by inhalation.

Species	$L(Ct)_{50}$ (mg min/l)	Exposure time (min)	Reference
Mouse	0.5	15-30	60
	0.87	10	17
	0.58	10	15
	0.34	10	7
Rat	2.0	2	40
	1.1	5	40
	0.78	10	40
	0.9	30	40
	1.3-1.8	5-10	46
Guinea pig	3.0	15-30	60
Rabbit	3.0	15-30	60
	>1.8	5-10	46
	<5.2	10	18
Goat	<1.6	5-10	46
Dog	3.0	10	45
Monkey	15-20	10	45
	15 ±	15	45
	21 ±	30	45
Man	1.5-2.9	75-90	49

posure times. Some of the variability in results is undoubtedly due to variation in particle size of the cadmium oxide. When dispersed from most incendiary munitions the median particle size is usually less than 1.0μ in diameter, but agglomeration of particles frequently takes place. This point has been particularly emphasized in the estimate of the $L(Ct)_{50}$ for man,⁴⁹ in which the elementary particles were less than 0.3μ in diameter, but in which the cloud actually consisted of large numbers of small agglomerates of 1.0 to 2.0μ in diameter, with a small number of agglomerates 40μ or greater in diameter. The $L(Ct)_{50}$ of 2.9 mg min/l was based on analytical Ct 's obtained in an experiment set up to duplicate conditions which resulted in two cases of fatal cadmium oxide poisoning in an industrial plant. There appeared to be every reason to believe that the concentration of cadmium oxide in the original accident was not greater than that obtained in the duplicate experiment, but the $L(Ct)_{50}$'s for rats and rabbits exposed in the duplicate experiment were about twice those previously obtained with arc-produced cadmium oxide fumes. The difference was attributed to the greater median particle size in the duplicate experiment and led to the hypothesis that the $L(Ct)_{50}$ for man might be as low as 1.5 mg min/l under con-

ditions where particles do not agglomerate.⁴⁹ It is worth noting that prior to the Canadian experiments⁴⁹ there was a tendency to assume that the toxicity of cadmium oxide for man would closely resemble that for the monkey, making the human $L(Ct)_{50}$ of the order of 15 mg min/l . This estimate would seem to be entirely too high. The true toxicity of cadmium oxide for man may be as great or greater than that of phosgene (see Chapter 3).

The toxicity of cadmium metal itself and of cadmium compounds other than the oxide by inhalation was tested at the UCTL. The results are shown in Table 2. So far as mice are concerned, there is con-

TABLE 2. Inhalation toxicities of cadmium compounds for mice.⁷

A = analytical concentration; N = nominal concentration.

Compound	Ct (as compound) (mg min/l)	Ct (as Cd) (mg min/l)	Avg. particle diameter (μ)	Mortality
Cadmium	0.38 A	0.38	<0.2	18/20
Cadmium	0.17 A	0.17	<0.2	18/19
Cd oxide	0.34 A	0.30	<0.2	$L(Ct)_{50}$
Cd chloride	2.3 A	1.4	<0.5	$L(Ct)_{50}$
Cd fluoride	1.8 N	1.2	?	0/20
Cd fluoborate	6.5 N	1.9	?	8/20
Cd fluosilicate	6.7 N	2.1	?	9/20
Cd sulfide	1.35 A	1.05	<0.3	5/20
Cd selenate	2.27 N	0.93	?	0/20
Cd nitrate	3.85 A	1.4	<0.5	$L(Ct)_{50}$
Cd phosphate	6.5 N	3.67	?	2/20

siderable variability in the toxicity of the different cadmium compounds even when dosages are calculated in terms of the cadmium content of the compound. It is also of interest that cadmium metal itself is more toxic than any of its salts. In this instance, the combination of cadmium with anions which are themselves toxic resulted in decreased rather than enhanced toxicity. Unfortunately it is not possible from the data to assess the effect of particle size on the different estimates of the $L(Ct)_{50}$'s but taking the results at their face value it would appear that cadmium oxide is the most toxic of the cadmium compounds. This fact is fortunate since the oxide is easily prepared in the field by the combustion of incendiary munitions containing cadmium metal.^{15,24,29,46}

11.2.3 Assessment of Value as a Chemical Warfare Agent

Cadmium appears to be a promising material for addition to incendiaries if toxicity as well as fire is

TABLE 3. Toxicity of selenium dioxide by inhalation.

Species	Ct (mg min/l)	Exposure time (min)	Mortality	Time to death (hr)	Reference
Mouse	2.30*	10	0/20		7
Rat	2.30†	10	0/6		7
Rabbit	5.89†	20	4/6	6, 6.5, 40, 132	27
	6.59†	10	4/6	3.8, 11, 13, 32	27
	13.18†	20	6/6	2.8, 3, 3, 5, 5.5, 8	27
Goat	5.89†	20	0/2		27
	6.59†	10	2/2	5.5, 84	27
	8.83†	30	3/4	6, 18, 130	27
	13.18†	20	2/2	4, 4.5	27

* SeO₂ dispersed by atomization of aqueous solution.

† SeO₂ formed and dispersed by detonation of Se/high-explosive mixture. Peak range of particle size 0.6 to 1.0 μ in diameter.

desired. The cadmium oxide which results from the combustion of cadmium metal in incendiary mixes is odorless and probably not irritating enough in the presence of smoke and burning matériel to be detected by odor.

Cadmium oxide smoke is brown in color, however, and may be detected by appearance after it has been used a few times. Cadmium chloride may also be dispersed from burning munitions^{18,19,22} and lethal concentrations may be obtained in mixtures which are indistinguishable in appearance from harmless screening smokes. Attempts have been made to disperse cadmium compounds by the explosion of munitions containing cadmium or its compounds,^{9e,10,50,55,64} but this method of dispersal is relatively inefficient because of the rapid agglomeration and settling of the cadmium particles.

One drawback to the use of cadmium in offensive warfare is the delay in appearance of toxic effects, since, as has been pointed out, dyspnea does not usually become severe until at least 12 hours after exposure. If, however, incendiary attacks are planned against industrial installations, large stores of matériel, cities, and the like, such targets are usually well beyond the front lines and a delay in the appearance of toxic effects can be readily accepted. From the available data it would appear that cadmium might play a very important role if a military requirement for toxic incendiaries should arise.

11.3

SELENIUM

A review of the toxicity of selenium as a potential industrial hazard appeared in 1938.⁶⁹ At that time it was known that selenium compounds were toxic when ingested and that hydrogen selenide was toxic on inhalation. On this basis it was predicted that soluble dusts such as selenium oxides (SeO₂, SeO₃,

H₂SeO₃, H₂SeO₄) and certain halogen compounds might be toxic because of the ease by which they could be absorbed from the lungs and gastrointestinal tract. These toxic dusts might be set up by the combustion of incendiary mixtures containing selenium or its compounds or by the detonation of explosives containing selenium. Hence selenium, like cadmium, was investigated as a possible chemical warfare agent.

11.3.1

Physiological Action

The action of selenium appears to be similar to that of cadmium, with the exception that the onset of toxic effects is more rapid after exposure to selenium. Goats and rabbits exposed for periods of 10 to 30 minutes to selenium oxide smoke showed dyspnea and tachycardia on removal from the exposure chamber. Animals receiving a fatal dose usually died within 24 hours and sometimes within 3 hours. On autopsy, pronounced pleural effusion and pulmonary edema were found, plus hemorrhages in the lungs, heart, and kidneys, and marked congestion of the glomeruli and spleen.²⁷

11.3.2

Toxicology

All workers agree that in absolute terms selenium is less toxic than cadmium. The toxicity of selenium oxide toward various species is shown in Table 3. Other selenium compounds were screened for toxicity at the UCTL without revealing any of greater interest or effectiveness than the oxide.⁷

11.3.3

Assessment of Value as a Chemical Warfare Agent

Selenium oxide smoke differs from cadmium oxide smoke in the following respects: (1) it is less toxic than cadmium oxide; (2) it is acrid and irritating to

the respiratory tract, whereas cadmium oxide is odorless and relatively nonirritating; (3) it is white, whereas cadmium oxide is brown; (4) it kills or disables more quickly than cadmium oxide. In contrast to cadmium, which was found to be most effective in incendiary munitions, selenium has been studied mainly in explosive-type munitions.^{27,31,33,34}

Since selenium oxide is white, it would not be detected by appearance alone if used in conjunction with ordinary screening smokes. On the other hand, its irritant properties might well lead exposed troops to mask promptly. Whereas its relatively rapid action as compared with that of cadmium is a desirable feature, it is doubtful whether this outweighs its lower absolute toxicity. The potential usefulness of selenium oxide as a chemical warfare agent cannot be accurately assessed on the basis of available information. If there is a future requirement for this type of agent, further experimentation, and particularly field trials, are in order.

11.4 NICKEL CARBONYL AND IRON CARBONYL

Nickel carbonyl, $\text{Ni}(\text{CO})_4$, was discovered in 1890, and iron pentacarbonyl, $\text{Fe}(\text{CO})_5$, in 1891. Both compounds can be made to dissociate into carbon monoxide and the pure metal under controlled conditions, and this reaction forms the basis for the commercial preparation of pure nickel (Mond process), a method which is in use to the present day. Iron carbonyl was more difficult to prepare than nickel carbonyl, the yield being only about 1 per cent of theoretical, so that the Mond process was not economical for the preparation of iron on a large scale. In the 1920's, however, iron carbonyl found some use in Europe as an "antiknock" for gasoline, and more recently it has been an important source of the pure, finely powdered iron which is used in powder metallurgy.

The toxicity of the metal carbonyls was recognized as early as 1891⁷⁰ and was extensively investigated and reported in 1907–1908.⁷⁰ Chemical warfare interest in the compounds arose primarily from two facts: (1) they are toxic enough to merit consideration as agents for use under certain circumstances where they might not be readily detected, and (2) they dissociate readily in contact with the active carbon of the gas mask, releasing four or five volumes of carbon monoxide per mole of carbonyl. The carbon monoxide is not absorbed by the canister

of the service gas mask. Therefore, the carbonyls provide an indirect way of bringing carbon monoxide into offensive chemical warfare.

11.4.1 Physiological Action

When iron or nickel carbonyl comes into contact with moist air, dissociation into carbon monoxide and a finely divided metallic salt takes place. This salt appears to be a hydrated basic carbonate of somewhat uncertain composition. Thus, when a person is exposed to an atmosphere into which iron or nickel carbonyl has been released, he breathes a mixture of varying proportions of the metallic carbonyl, carbon monoxide, and a dust of finely divided metallic salt. What part each of these components may play in the toxicological picture will be discussed, but for the moment the discussion will be limited to the overall effects of inhalation of an atmosphere known to contain originally iron or nickel carbonyl. Since the physiological action of the two compounds is essentially the same, they will be discussed together.

Armit in 1907⁷⁰ described the sequence of events in human cases of nickel carbonyl poisoning as follows:

... immediately after having been exposed to air containing plant-gas there was giddiness, and at times dyspnea and vomiting. These symptoms passed off rapidly as soon as the patients were brought into the fresh air. After 12 to 36 hours the dyspnea returned, cyanosis appeared, and the temperature began to be raised. Coughing with more or less blood-stained sputum occurred on the second day or later. The pulse rate became increased but not in proportion to the respiratory rate. Delirium of varying types frequently occurred, and a variety of other signs of disturbance of the central nervous system was noted. Death took place in the fatal cases between the 4th and 11th days. The chief changes found post mortem were hemorrhages in the lungs, edema of the lungs, hemorrhages in the white matter of the brain (in one case this was very extensive), while some doubt exists as to whether any blood changes were present.

This sequence of events parallels closely that of a fatal case of human nickel carbonyl poisoning described in 1934,⁷² in which death occurred on the seventh day following exposure. The reaction of mice, rabbits, cats, dogs, guinea pigs, and goats is similar to that of man.^{9a,b,c,d,10,70}

If masked troops are exposed to air containing iron or nickel carbonyl, the carbonyl is catalytically decomposed in contact with the active carbon of the mask, leaving the finely divided metallic salt and carbon monoxide. The metallic dust is efficiently retained by the particulate filter of the service mask,

but the carbon monoxide passes through. When mice were exposed to atmospheres which had originally contained iron or nickel carbonyl but which had then been passed through active carbon, deaths which occurred were entirely due to carbon monoxide and bore no similarity to those resulting from exposure to the carbonyls *per se*.^{9a,b} The effect of the carbonyls on masked troops, therefore, is quite different from their effect on unmasked troops.

11.4.2 Toxicology

The toxicity of iron and nickel carbonyl by inhalation is shown in Tables 4 and 5. These data leave

TABLE 4. Toxicity of iron pentacarbonyl.
All concentrations were nominal.

Species	Ct (mg min/l)	Exposure time (min)	Mortality	Reference
Mouse	73 ± 24	10	$L(Ct)_{50}$	13
	75	10	49/50	7
	70	10	6/10	7
	61	10	4/10	7
Rabbit	90	45.5	lethal	70

TABLE 5. Toxicity of nickel carbonyl.
A = analytical concentration; N = nominal concentration.

Species	Ct (mg min/l)	Exposure time (min)	Mortality	Reference
Mouse	1.7 A	10	$L(Ct)_{50}$	7
Rat	5.1 A	10	1/5	9a
Guinea pig	5.1 A	10	0/1	9a
Rabbit	5.1 A	10	0/1	9a
	73-76 N	50.5	64/77	70
Dog	5.1 A	10	0/1	9a
	217 N	75.5	lethal	70
Cat	5.1 A	10	0/1	9a
	241 N	75.5	25/30	70

much to be desired, but the picture is obscured partly because of the uncertainty as to the composition of the gas mixture breathed (the relative proportions of carbonyl, carbon monoxide, and metallic salt), and partly because some of the concentrations are nominal and hence may be grossly inaccurate. It would appear from the data shown, however, that iron carbonyl is less toxic to mice and rabbits than nickel carbonyl.

Carbon monoxide produced in air or *in vivo* by the dissociation of the carbonyls would, of course, combine with hemoglobin to form carbon monoxide

hemoglobin, and this in turn if produced in sufficient quantity leads to death by asphyxiation. There is evidence, however, that carbon monoxide does not play a significant part in death resulting from exposure to a minimum lethal dose of the carbonyl, since: (1) animals killed with nickel carbonyl had at the time of death only 5 per cent carbon monoxide hemoglobin; (2) animals whose blood contained 32 per cent carbon monoxide hemoglobin from pre-exposure to pure carbon monoxide actually lost carbon monoxide during exposure to a fatal dose of nickel carbonyl; (3) iron carbonyl yields 1.25 times as much carbon monoxide per mole as nickel carbonyl, but is less toxic than the latter; (4) the pathology resulting from exposure to minimum lethal doses of iron or nickel carbonyl does not resemble that produced by carbon monoxide.⁷⁰ Thus, carbon monoxide does not play an important role in poisoning from minimum doses of the carbonyls, although, if larger quantities of the carbonyls are present, it may contribute to the pathological picture.

If carbon monoxide effects are of no consequence in minimum lethal dosages of the carbonyls, the toxic effects must be due to inhalation of either the unchanged carbonyl or the finely divided metallic salt. It was observed⁷⁰ that when nickel carbonyl was released into a gas chamber a smokiness rapidly appeared as a result of the formation of a cloud of small particles of nickel salt. Fundamental studies of the rate of breakdown of nickel carbonyl in the presence of air and moisture led to the hypothesis that nickel carbonyl would be completely dissociated either before or soon after reaching the alveoli of the lungs.⁷⁰ On this basis, the toxicity of nickel (or iron) carbonyl was attributed entirely to the metal and to the fact that the metal is introduced into the lungs in such a fine state of subdivision that it readily penetrates to the alveoli.⁷⁰ By tissue-staining techniques the presence of nickel was demonstrated in the mucous membranes of the respiratory tract and in the free surfaces and tissue immediately surrounding the surface of the bronchi, bronchioli, and alveoli. Staining was intense at the free edges and diffuse in the neighboring tissues.⁷⁰

If the toxicity of the carbonyls is entirely due to the metallic part, the parenteral administration of the metal in question or its salts should produce essentially the same effects as the inhalation of the carbonyls. This experiment was performed,⁷⁰ and, when finely divided nickel salts were injected subcutaneously or intraperitoneally in guinea pigs, rab-

bits, or cats, in doses which were calculated to be of the same order of magnitude as those found lethal for the carbonyl by inhalation, the course of the poisoning and post-mortem changes in lungs, brain, and adrenals were said to be similar to those found in nickel carbonyl poisoning. The intravenous injection of nickel carbonyl in rabbits has also been found to produce lung edema and damage to the lung capillaries.¹⁰ The injection of iron or its salts is said to have produced symptoms and pathological changes similar to those resulting from the inhalation of iron carbonyl.⁷⁰

In rabbits the lethal dose of nickel by subcutaneous injection was approximately 7.5 mg/kg, by intraperitoneal injection about 7 mg/kg, and by inhalation^a of the carbonyl about 3 to 4 mg/kg.⁷⁰ With cats the lethal dose of nickel was about 12.5 mg/kg by subcutaneous injection as compared with about 8.5 mg/kg by inhalation of nickel carbonyl.^a The lethal dose of iron when given by intraperitoneal injection to rabbits was about 20 mg/kg. The slightly greater apparent toxicity of the carbonyls over the metallic salts was attributed to the fact that the lungs offer a more favorable surface for rapid absorption than the sites of subcutaneous or intraperitoneal injection.⁷⁰ However, the calculated inhalation dosages in these experiments were based on nominal concentrations of the carbonyl, and it is possible that the true inhalation dosages were very much lower and that the agreement between the lethal dose of carbonyl by inhalation and of the metallic salts by

^a Inhalation concentrations were nominal, and hence inhalation dosages may have been greatly overestimated.

injection is fortuitous. The question of whether the carbonyls are more toxic than the metallic residue liberated by their decomposition can only be answered by studying the effect of breathing such metallic dusts uncomplicated by the presence of either carbon monoxide or the unchanged carbonyl. Nevertheless, a qualitative (and perhaps quantitative) correspondence is reported between the effects produced by the inhalation of the carbonyls and those produced by injection of the corresponding metallic salts.

11.4.3 Assessment of Value as Chemical Warfare Agents

The intrinsic toxicity of the carbonyls is probably too low to recommend them as primary chemical warfare agents except under very special conditions. If used as sources of carbon monoxide for mask-breaking operations, they might have limited application in the attack of enclosed fortifications, but more efficient mask-breakers are known (e.g., cyanogen chloride, see Chapter 2). It should be borne in mind, however, that the carbonyls are inflammable, miscible with petroleum products, and thus suitable adjuvants to flame thrower fuels.¹⁴ In flame thrower operations there are frequently situations such as in the attack of enclosed fortifications where considerable advantage could be gained from the high carbon monoxide concentration and toxic dust resulting from the incomplete combustion of fuels fortified with the carbonyls, and this aspect of their use undoubtedly merits careful consideration and further investigation.

Chapter 12

RICIN

12.1 INTRODUCTION ^a

THE ISOLATION of the toxic protein ricin from castor beans and the investigation of its properties from the standpoint of assessment as a possible chemical warfare agent were studied under Division 9 of the National Defense Research Committee [NDRC] during the period 1942–1945. Earlier work by British investigators had shown that ricin (commonly coded as “W”) could be dispersed as a particulate, nonpersistent, toxic cloud by explosion of bombs containing a suspension of ricin in carbon tetrachloride. Notable progress was made by NDRC investigators in all phases of the work with ricin.

Processes for the extraction of ricin from castor beans and cold-pressed castor bean pomace were the subject of laboratory and pilot plant studies. During the laboratory investigations the protein was crystallized; the crystals were not completely homogeneous but represent the purest ricin so far obtained. The pilot plant development culminated in a process of extraction of castor bean pomace with water and purification of the toxin by two precipitations with sodium sulfate. A water solution of the purified toxin was spray-dried to give a dry product with a mass median diameter of 6–8 μ . This was air-ground to give “dispersible ricin” with a mass median diameter of 2.5–3.5 μ , which was approximately half as toxic (by injection) as crystalline ricin.

Early work on the physiological action of ricin resulted in the development of a bioassay procedure in mice which was used to determine the toxicity of various ricin preparations. Later studies investigated the inhalation toxicities of various ricin preparations, which are a function of the intrinsic toxicity of the material and the particle size distribution in the inhaled particulate cloud.

Toxoids have been prepared from ricin by various means, most successfully by treatment with formalin. The toxoid has been used to produce in horses and rabbits antiricin serums. These have been purified and concentrated as antiricin globulin fractions that were made available for therapy in case of accidental exposure. Immunization against ricin appears impractical at present because of the short duration of

passive immunity in animals, and the toxicity and local necrotizing action of toxoid preparations available for use in inducing active immunity.

The detection and assay of ricin in the field is a difficult problem. Sensitized guinea pigs afford the most sensitive, rapid, and specific means of detection through their anaphylactic response. Hemagglutination and precipitin tests have been used; chemical tests are less specific. Determination of particle size distribution forms an important part of the assessment of ricin and all other particulate clouds (see Chapter 15). Field trials employing these analytical means and animals exposed to the clouds to determine toxicity have been conducted to evaluate ricin as a war gas and determine the efficiency of various munitions for its dispersal.

Ricin is most efficiently dispersed from small high explosive-chemical bombs as a suspension in carbon tetrachloride of the most finely divided material available. On the basis of airplane stowage such bombs are estimated to be seven times as effective as bombs charged with phosgene.

Processing all of the castor beans used in this country (based on 1941–1944 consumption) by the optimum procedure based on pilot plant experience would yield approximately 1,000 tons of dispersible ricin annually at a cost of about \$13 per pound. This is a significant quantity of a material which might be used as a unique nonpersistent agent in gas warfare, difficult to detect and disturbing to morale because of its delayed toxic action. Ricin has served as a model substance, presenting problems in preparation, protection of personnel, detection, assay, and dispersal similar to those presented by other materials investigated in the field of bacteriological warfare.

Some minor duplications appear in the subsections of this chapter, which were written by different authors.

12.2 PREPARATION OF RICIN ^b

The isolation from castor beans of products containing the toxic principle known as ricin has been recorded many times in the open literature within the past 60 years.⁶⁴ During World War I ricin was

^a By Arthur C. Cope.

^b By Joseph Dec.

examined as a candidate chemical warfare agent and its preparation was studied.²⁴ The investigation of the preparation and properties of ricin pertinent to its use as a chemical warfare agent was renewed in Great Britain³³ early during World War II and in this country under NDRC Division 9 during the fall of 1942.^{1,4}

The objective of developing a process for the large-scale production of ricin in a form suitable for dispersion from munitions was attained.¹¹ During the course of this development about 3,800 lb of material was produced on a pilot plant scale.^{1,11,35} Also of considerable importance was the preparation of ricin in a crystalline form² for the first time.

A complete review of the great number of products containing ricin whose preparation has been recorded both in the open and classified literature is beyond the scope of this chapter. Emphasis is placed herein on the products studied most extensively during World War II and on the studies leading to their preparation. These include crystalline ricin; two products used in field trials with munitions, 470 BM 199 and L703; and the material used for the preparation of toxoid, B1. A process for the large-scale production of ricin is outlined.

12.2.1 Crystalline Ricin

The isolation during the late summer of 1943 of the material responsible for the toxicity of crude ricin preparations in crystalline form was a signal achievement.² Neither the first crystals isolated nor any of the crystalline materials subsequently prepared^{12,15} could be shown to be single substances.^{2,15} Since the crystalline material was the most toxic fraction ever isolated from crude ricin, studies were initiated to determine its physical and chemical properties, composition, and physiological behavior.

PROPERTIES

The crystalline material is a protein of the globulin type,^{2,15} although the crude toxin shows albumin-like solubility behavior. Repeated crystallizations fail to increase its toxicity,^{2,12} which has been assayed to be 500⁹ and 750¹² TU (depending on the method of evaluating TU; for definition of the toxicity unit known as TU see Section 12.5). The protein is soluble in acid or alkaline solution, is least soluble in the range of pH 5.0 to 8.0,^{2,12,15} and is more soluble in the presence of other proteins.^{2,15} Its ultraviolet light absorption spectrum is similar to that of a typical protein,^{2,15} and it has a specific optical rotation of

−26.³ Ultracentrifuge and electrophoresis measurements showed the material to be fairly homogeneous.^{2,3} However, solubility measurements indicated the crystalline material to consist of a solid solution of more than one component.^{2,15} On the basis of sedimentation and diffusion studies the molecular weight has been estimated at 36,000² and 77,000.³ The rate of denaturation of the crystalline material in aqueous solution to a product insoluble at pH 5.1 has been determined at 65.3, 71.5, 78.1, and 86.5 C, and from pH 2 to pH 11.¹⁵

The chemical composition of the crystalline material has been investigated, but not exhaustively. Evidence was obtained that the *d*-amino acid content of an acid hydrolyzate of the toxin cannot be more than 3 per cent.¹⁶ On a moisture- and ash-free basis a sample of three times crystallized ricin was found to contain 16.23 ± 0.4 per cent nitrogen.¹⁵ From the titration curve of crystalline ricin in water and in the presence of 8 per cent neutral formaldehyde the numbers of basic, amino, imidazole, and carboxyl groups were deduced.¹⁵ The amide nitrogen, alkali labile ammonia, hydroxyamino acid, arginine, histidine, aspartic acid, and glutamic acid¹⁵ contents have been determined by chemical analysis. The amino acid analyses referred to account for 50 per cent of the weight of the protein and 60 per cent of the nitrogen. The protein was found to contain 1.34 per cent sulfur and less than 0.1 per cent phosphorus.

Preliminary to the first successful crystallization, ricin-sodium sulfate cake, an amorphous product (described in Section 12.2.5), was fractionated with ammonium sulfate at pH 6.8 to concentrate the toxin.² The moist solid was dissolved in a minimum of water and allowed to stand at 5 C. A granular precipitate formed, which gradually became crystalline on standing for several weeks. The crystals were isolated, suspended in water, and dissolved by the addition of a little hydrochloric acid. The solution was adjusted to pH 6.8 and allowed to stand at 5 C. Recrystallization was complete in 2 or 3 days.

Crystallization procedures more rapid and productive than the original method were developed.^{12,15} Two extractions of the ricin-sodium sulfate cake with 10 parts of sodium sulfate solution (19 g Na₂SO₄/100 ml H₂O) were found to leach away many of the gummy low molecular weight impurities without appreciable loss of the toxin.¹⁵ One useful procedure¹⁵ involved extracting the residue with water and allowing the solution to stand overnight in a

refrigerator. The precipitate which formed was removed and dissolved in water with the aid of a little acid. The solution was neutralized, seeded with a few crystals, and stored in the cold for several days to yield a crystalline precipitate, which was separated and recrystallized. A modification of this procedure was performed starting with 1 kg of ricin-sodium sulfate cake.¹² The yield was about 70 g of crystalline material, which is 7 per cent by weight or about 35 per cent of the toxin content of the starting material. Recrystallization was complete in 12–36 hours with an 85–90 per cent recovery.

A 2- to 2 $\frac{1}{4}$ -hour dialysis of a 20 per cent aqueous solution of ricin-sodium sulfate cake also served to remove the low molecular weight impurities.¹² The dialyzed solution after filtration and standing in a refrigerator yielded a crystalline precipitate. The percentage yields were comparable with those obtained in the procedure involving preliminary purification with sodium sulfate solution.

In an attempt to obtain a pure sample of ricin for an absolute standard, 60 g of 4 times crystallized material was extracted 25 times with 0.1 per cent sodium sulfate solution at pH 7.0 and 10 C.¹² The residue of about 6 g was recrystallized. Solubility studies on this product have not yet been made. Although the product is probably the purest sample of ricin obtained thus far, its allergen content has been estimated at about 0.1 per cent on the basis of animal assay.¹²

Numerous experiments were performed in the study of the crystallization of ricin which led to the procedures just described.^{2,12,15} Flotation-purified ricin and the ball-milled and hammer-milled products (described in Section 12.2.5) were less satisfactory than ricin-sodium sulfate cake as the starting material;¹² however, crystalline material has been obtained from flotation-purified ricin.¹⁵ Although a short dialysis of a solution of ricin-sodium sulfate cake is satisfactory for the preliminary purification, exhaustive dialysis is not.¹² Some impurities can also be removed by adsorption on Celite or floridin.¹²

12.2.2

Amorphous Ricin

Studies on the preparation of amorphous ricin have been extensive and a great number of products of varying properties and content of toxin, nontoxic protein, protease, and salt have been obtained.^{4,9,15,34} Crude ricin is soluble in water and dilute salt solutions. In the dry state the products are normally stable at room temperature and denatured at ele-

vated temperatures.^{4,30a,33} The stability decreases with increasing moisture content.^{4,30b} Aqueous solutions are less stable than the dry product at both room and higher temperatures.^{4,15,33}

STARTING MATERIAL FOR PREPARATION OF RICIN

Samples of ricin prepared from castor beans of different sizes and colors seem to be identical in physical, chemical, and immunological properties.^{1,3,4} The maximum variation in toxin content of the different beans which were examined in one laboratory was 34 per cent.⁴

The beans contain about 50 per cent oil and the toxin is best isolated after removal of a substantial portion of this oil. The castor bean pomace which is obtained in the laboratory using a Carver press⁴ or in industry using a hydraulic press¹¹ contains about 15 per cent oil and is satisfactory for the aqueous extraction of the toxin. A pomace containing 1–2 per cent oil can be prepared by extraction of either ground castor beans or cold-pressed pomace with suitable organic solvents.^{4,34} If desired, the bean hulls can be removed from the pomace by flotation in organic solvents.^{4,15}

Hydraulic-pressed castor bean pomace is prepared commercially by castor oil producers. In one of the commercial processes the castor beans are ground, heated to about 60 C, and pressed.¹¹ This cold-pressed pomace is recommended as the starting material for the large-scale production of ricin.^{4,11} Commercially, this product is extracted four times with heptane at 82–87 C to obtain the remaining castor oil and then blown with steam to recover the residual heptane.²² The latter step also serves to detoxify the pomace, which is sold as fertilizer. Tests on a laboratory and pilot plant scale showed that no appreciable detoxification occurs during the extraction with hot heptane.^{4,11} Efforts to find an economical procedure for recovery of the residual heptane without detoxification of the pomace were unsuccessful.¹¹ Extraction of the cold-pressed pomace with water at pH 3.8 to remove the toxin and subsequent solvent extraction yielded castor oil containing free fatty acid.¹¹

EXTRACTION OF TOXIN FROM BEAN MEAL

Among the solvents which have been used to extract the toxin from castor beans or the pomace are water, dilute salt solutions, glycerol, ethylene glycol containing a little water, and diethylene glycol containing a little water. Water and dilute salt solutions are the most efficient and economical extractants for

the toxin.⁴ Ten per cent saline is slightly more effective than water; however, it also dissolves more nontoxic material, most of which is coagulable protein.^{4,15} About $3\frac{1}{2}$ –4 parts of water at pH 3.8 to 1 part of pomace seems to be most satisfactory. Less nontoxic protein is dissolved at pH 3.8 than at pH 7.0 and filtration is accomplished more easily.⁴ Extraction at temperatures approaching 70 C proceeds more rapidly than at room temperature but is accompanied by denaturation of the toxin.⁴

ISOLATION OF TOXIN FROM AQUEOUS EXTRACT OF POMACE

The toxin may be precipitated from the aqueous extract of pomace by nonaqueous solvents, by picric acid and similar precipitants, and by inorganic salts. Organic solvents such as alcohols and ketones precipitate the toxin from aqueous solution but rapidly denature it at room temperature. At temperatures below 0 C acetone has been used to precipitate and wash the toxin.^{4,34} The use of ammonium sulfate,^{4,34} sodium chloride,^{4,11,15} and sodium sulfate^{3,4,11,15} for precipitating and fractionating the toxin has received considerable study. Ammonium sulfate has been used for precipitating the toxin on a pilot plant scale.³⁵ Sodium sulfate is now regarded as the best precipitant. It is superior to sodium chloride because it gives better fractionation, is less sensitive to changes in pH, and precipitates the toxin more completely.^{4,11,15} The importance of temperature control during precipitation, filtration, and drying when sodium sulfate is used have been studied.⁴ Many data on the salting out of the toxin with different amounts of sodium sulfate and at different pH have been obtained.^{3,4,11,15} These data were useful in the development of the process for the large-scale production of amorphous ricin (Section 12.2.3).¹¹

Better yields of the toxin have been obtained in the laboratory than in the pilot plant.^{4,11,15} In the methods preferred by some investigators^{4,15} slightly less sodium sulfate is used than in the proposed large-scale process and the first precipitation is performed at pH 3.8. In one laboratory run,¹⁵ during which the isolation of the toxin was followed by chemical analyses and toxicity determinations, the product amounted to 2.3 per cent of the pomace weight. It contained 10.4 per cent nitrogen and 32.5 per cent inorganic material and had a TU value of 196. Of the toxicity present in the extract, 92 per cent was recovered. The product obtained at a similar stage in the pilot plant process amounted to 1.4 per cent

of the pomace weight. Procedures involving a single precipitation of the toxin with sodium sulfate yielded in the laboratory products with TU values above 200,^{4,15} but these methods were not satisfactory on a pilot plant scale because of operational difficulties.^{4,11}

Removal of water by lyophilization of solutions of partially purified ricin yields products of good appearance and stability.^{4,11} Dialysis can serve to remove much organic and inorganic impurity and in neutral solution leads to a precipitate of amorphous ricin.⁴

COMMUNITION OF AMORPHOUS RICIN

Since the toxicity by inhalation of ricin aerosols increases with decreasing particle size,⁹ considerable effort was directed toward developing a method to produce finely divided, readily dispersible material without concomitant denaturation of the toxin. The process involving spray drying and air grinding of partially purified ricin was the best solution found to the problem.¹¹ Prior to this solution an appreciable number of other methods were considered and explored.^{8,11}

The particle mass median diameter of freshly precipitated crude ricin is 1–2 μ , but as the moist filter cake is dried the particles agglomerate. The final precipitation was performed under various conditions with the objective of obtaining a product that could be ground readily to fine particles.¹¹ Among the conditions investigated were temperature of precipitation, agitation during precipitation, addition of sodium sulfate as a dry powder or from saturated solution, variation in amounts of sodium sulfate used, addition of colloids, addition of seeding agents, addition of nonionic wetting agents, and transfer of the freshly precipitated product to a volatile liquid. A 2-hour ball-milling test was used for comparing all of the samples obtained in this series of tests. None of the experimental products showed significantly superior grinding properties. Lyophilization of solutions of partially purified ricin proceeds without detoxification to give a friable mat-like solid.^{4,11} Ball-milling the solid reduced the particle size to a mass median diameter of 6 μ in 33 per cent less time than that required with precipitated air-dried material.¹¹ The detoxification which accompanied the ball-milling was 20 per cent less than with precipitated air-dried material.¹¹ Lyophilization of a pomace extract yielded a gummy product.¹¹

Flotation-purified ricin-sodium sulfate cake (described in Section 12.2.4) was used in ball-milling,

colloid-milling, and hammer-milling experiments.¹¹ Hammer-milling gave products with particle mass median diameters no smaller than 20 μ . Colloid-milling was even less effective. For about a year ball-milling appeared to be the most promising method for obtaining a finely divided material, and this method was investigated intensively.^{11,48,51} The optimum conditions using an Abbé 4-jar mill fitted with 1 $\frac{1}{4}$ gallon "specimen" type porcelain jars, which were found to give a 4- to 6- μ product, involved (1) steel balls for the milling, (2) low milling temperature (-20°C), (3) low moisture content ricin, and (4) milling a suspension of ricin in carbon tetrachloride.¹¹ Factors affecting the ball-milling that were studied included the vehicle, grinding media, temperature, time, and moisture content of the amorphous ricin.¹¹ The ball-milling time necessary to give a 4- to 6- μ product was proportional to the load of ricin in the jar, 1 lb of ricin requiring 8 hours. Ball-milling a high moisture content material at room temperature or in the dry state resulted in more denaturation of the protein than otherwise. Even under the above optimum conditions at least 50 per cent detoxification accompanied ball-milling the material to a mass median diameter of 4–6 μ .¹¹ The toxicity loss was reduced somewhat by drawing off the fine particles as they were formed.⁵¹

A combination of spray drying and air grinding was found to give a product with a mass median diameter of 2.5–3.5 μ with little denaturation of the starting material.¹¹ A spray dryer was constructed and conditions for its operation investigated.¹¹ Factors such as type of nozzle, solution concentration, atomizing air pressure, drying rate, drying temperature, and amount of drying air were studied. Under optimum conditions at an operating rate of 1 lb of product per hour the product has a particle mass median diameter of 6–8 μ and is 95 per cent soluble in water. The spray-drying process is superior to the ball-milling method from the standpoints of low toxicity loss, processing time required, safety, and cost.

Several types of air-grinding equipment were investigated for the comminution of spray-dried ricin.¹¹ A grinder previously developed by the Eagle Pencil Company was found to be the best of the types examined. Optimum conditions for its operation in a low humidity room were determined. Under optimum conditions the product with a particle mass median diameter of 2.5–3.5 μ and a TU value of 225 is obtained at a rate of 1 lb per hour. A reduction in

toxicity of about 5 per cent accompanies the air-grinding operation.

12.2.3 A Process for the Production of Finely Divided Ricin¹¹

On the basis of considerable laboratory and pilot plant data a process for the production of finely divided ricin at the rate of 26 lb per day has been outlined. The equipment and manpower necessary for this scale of operations have been determined. The process involves extraction of the toxin with water from castor bean pomace, two precipitations of the toxin by addition of sodium sulfate, spray drying of a solution of the partially purified toxin, and air grinding of the spray-dried material. It was estimated that the cost of such a pilot plant would be approximately \$125,000 and that the cost of production at the 26 lb per day rate would be about \$16 per pound. The cost of operating a plant to produce 2,000 lb of "dispersible ricin" daily was estimated to be approximately \$13 per pound of product. The product has a particle mass median diameter of 2.5–3.5 μ and a toxicity value of 225 TU. The yield is 0.65 per cent based on the pomace and would have amounted to about 1,050 tons annually during the years 1941–1944 if the castor beans crushed in this country during those years had been processed by this method.¹¹ Reworking of the by-products from the spray-drying and air-grinding operations and reuse of the nitrogen-containing sodium sulfate separated in the flotation step should increase the yield to about 0.85 per cent.

STARTING MATERIAL

The starting material for this process is commercially available hydraulic-pressed castor bean pomace which has not been solvent-extracted to remove the residual oil and subsequently steamed. The pomace produced by one company averages 8.0–8.5 per cent moisture, 14.0–16.0 per cent oil, and 4.6–5.0 per cent nitrogen. The pomace is ground in a hammer mill prior to extraction.

EXTRACTION OF POMACE

The recommended conditions for extraction of the toxin from pomace are as follows:

Water for extraction	350 per cent of pomace weight
pH	3.8 ± 0.1
Acid to adjust pH	5 per cent H_2SO_4
Agitation time	60 minutes (not critical)

Temperature of extraction	25 C
Filtration	Continuous vacuum filter
Filter aid	7 per cent of pomace weight
Water for washing	50 per cent of pomace weight

Under these conditions at least 97 per cent of the extractable toxin is recovered. The amount of water used is the minimum necessary to produce a slurry that can be handled satisfactorily in plant scale equipment. Sulfuric acid is preferred over hydrochloric acid because of lower cost and lower corrosion rate. Continuous vacuum filtration at a higher *pH* is not possible because of the changed physical character of the slurry. The filter aid is necessary to insure a satisfactory filtration rate. Filtration with the vacuum filter proceeds about 30 times faster than with a recessed plate type filter.

FIRST PRECIPITATION AND FILTRATION

The optimum conditions for precipitation of the toxin from the extract and subsequent filtration were determined to be as follows:

Salt usage	20 per cent Na_2SO_4 , based on filtrate weight
<i>pH</i>	7.0
Alkali to adjust <i>pH</i>	12 per cent Na_2CO_3
Temperature	25 C
Time of precipitation	20 minutes
Filtration	Continuous vacuum filter
Filter aid	4 per cent of slurry weight
Wash solution	20 per cent of 16.7 per cent Na_2SO_4 , based on weight of extract

Under these conditions 50 per cent of the total nitrogen in the extract remains in solution and is eliminated in the filtrate, whereas less than 2 per cent of the toxin is lost. Precipitation at *pH* 7-8 was found to remove 6-10 per cent more nontoxic nitrogen than at *pH* 3.8. Increasing the temperature from 25 to 35 C and varying the precipitation time from 15 to 60 minutes showed no appreciable effects. The rate of filtration with a vacuum filter was $3\frac{1}{2}$ times that with a plate and frame filter press, filter aid being necessary to obtain a satisfactory filtration rate in both cases.

A full-scale pilot plant run was made to determine whether a single precipitation process would give a product suitable for spray drying.¹¹ Filter aid was not used, because previously it had been found not

possible to reduce the sodium sulfate content of a product containing filter aid by a process involving flotation in carbon tetrachloride. Despite the absence of filter aid, which made the filtration very slow, the dried product separated very poorly in carbon tetrachloride. The product, which amounted to 1.0 per cent of the original pomace, contained 11.0 per cent nitrogen and had a toxicity value of 200-250 TU. The operational difficulties encountered indicated this one-step process to be unsatisfactory on a pilot plant scale.

SECOND EXTRACTION AND FILTRATION

The optimum conditions for extraction of the toxin from the ricin-sodium sulfate-guhr moist filter cake were found to be as follows:

Water for extraction	300 per cent of wet cake weight
<i>pH</i>	3.8 ± 0.1
Acid to adjust <i>pH</i>	5 per cent H_2SO_4
Filtration	Continuous vacuum filter
Water for washing	25 per cent of slurry weight

An additional 10 per cent (based on the pomace extract) of nontoxic nitrogen is removed during this operation. The *pH* was varied from 3.8 to 9.0, and it was found that 5 per cent (based on *pH* 3.8 extract) more nontoxic nitrogen is removed at *pH* 3.8 than at *pH* 9.0. The filtration is very rapid because of the large amount of filter aid present.

SECOND PRECIPITATION AND FILTRATION

The recommended conditions for the second precipitation of the toxin and subsequent filtration are as follows:

Salt usage	20 per cent Na_2SO_4 , allowance being made for the sodium sulfate in the filtrate
<i>pH</i>	7.0
Alkali to adjust <i>pH</i>	12 per cent Na_2CO_3 , or more dilute
Temperature	25 C
Time of precipitation	45 minutes
Filtration	Plate and frame filter press
Filter aid	None
Washing	None

Drying of the filter cake can be accomplished in 6-10 hours using a three-section hot-air dryer operated at successively increasing temperatures from 55 C to 75 C. The dried product is given a slight

grind, passed through a five- to ten-mesh screen, and slurried in five parts of carbon tetrachloride. The toxin is removed from the surface of the mixture and dried. The sodium sulfate which settles to the bottom is used in the precipitation steps.

A quantity of partially purified ricin was produced by the process outlined except that the product was dried at 50 C. The product was obtained in 0.85 per cent yield based on the pomace, contained 13.0 per cent nitrogen, and had a TU value of 250–300.

Pilot plant tests indicated that a minimum of 20 lb of sodium sulfate is necessary to prevent loss of toxin. Approximately 3 per cent more nontoxic nitrogen is removed at pH 7.0 than at pH 3.8. Operation at 35 C instead of 25 C removes 2 per cent more nontoxic nitrogen, but about 2 per cent more toxin is lost. Since filter aid cannot be employed in this step, the use of a vacuum filter, which requires filter aid, is not possible. However, the physical character of this second precipitate permits a satisfactory filtration rate with a plate and frame filter press. Washing the filter cake with sodium sulfate solution (19.5 lb Na_2SO_4 /100 lb H_2O) does not result in sufficient purification to warrant a washing operation.

The utility of a third precipitation of the toxin with sodium sulfate was investigated. No appreciable purification was obtained without concomitant loss of toxin.

SPRAY DRYING AND AIR GRINDING

A 20 per cent aqueous solution of the above flotation-purified product is spray-dried under certain prescribed conditions at the rate of 1 lb per hour to give solid particles, which are 95 per cent soluble in water and have a mass median diameter of 6–8 μ . The solution for the second precipitation step can be spray-dried but it would contain about 50 per cent sodium sulfate. It was not found possible to separate the sodium sulfate from a spray-dried product by flotation in carbon tetrachloride.

Air grinding of the spray-dried material is carried out under certain defined conditions in an air grinder, previously developed by the Eagle Pencil Company, at a rate giving about 1 lb of product per hour. This operation reduces the toxicity of the material about 5 per cent. The product has a particle mass median diameter of 2.5–3.5 μ and a TU value of 225.

12.2.4 Four Amorphous Ricin Products

The four amorphous ricin products described in this section are of particular interest because of the

considerable extent of studies performed with them. The preparations known as (1) ricin-sodium sulfate cake, (2) 470 BM 199, and (3) L703 represent successive stages in the development of an amorphous ricin product in a form suitable for dispersion from munitions, and (4) B1 was used for the preparation of a toxoid.

RICIN-SODIUM SULFATE CAKE¹

A total of 1,550 lb of the product known as ricin-sodium sulfate cake was prepared on a pilot plant scale at the request of NDRC Division 9,¹ and an additional 2,000 lb was prepared for the Canadian government.¹¹ The method used in these operations, which was based on a procedure previously developed in another laboratory,⁴ utilized the facts that crude ricin is soluble in water and insoluble in saturated aqueous solutions of sodium chloride and sodium sulfate. Subsequent studies resulted in a marked improvement in the method of preparation (Section 12.2.3).¹¹

Castor beans were the starting material and an Anderson expeller was used for expressing the oil from the beans. From each ton of beans was obtained 810 lb of #3 grade castor oil. The expeller cake, which contained 13.1 per cent oil, 11.2 per cent moisture, and 4.6 per cent nitrogen, was ground in a hammer mill. Three parts of water at 15–20 C were mixed with the ground cake, the mixture agitated for 1 hour, the pH adjusted to 3.8 ± 0.1 with 5 per cent hydrochloric acid, and the slurry filtered in a plate filter press.

The filtered extract at pH 3.8 ± 0.1 and 17 C was saturated with sodium chloride to precipitate the toxin. The precipitate was separated by filtration, sufficient guhr being used to insure a satisfactory filtration rate. A sample of dried filtered cake was found to contain 33 per cent guhr and 33 per cent sodium chloride. The wet precipitate was mixed with five parts of water and the mixture adjusted to pH 8.0 with 5 per cent sodium hydroxide solution. The mixture was agitated for 1 hour and then filtered to remove guhr and other impurities. The filtrate was saturated with sodium sulfate, allowance being made for the sodium chloride present. The mixture was adjusted to pH 7.0 and then filtered at 35–40 C. The filter cake, about 1 inch thick, was dried in trays for 60–72 hours at a maximum temperature of 60 C and then packaged.

About 55 per cent of the toxicity available in the starting material was present in the ricin-sodium sulfate cake. The TU value^{1,9} of the cake was 100–

125. Analysis of the product showed 4.4 per cent moisture, 46.6 per cent ash, and 8.6 per cent nitrogen, of which 97 per cent was soluble and 45 per cent coagulable.⁹ Electrophoretic and ultracentrifugal studies indicated the cake to consist of several components with toxicity and hemagglutinating power associated with only the B1 fraction.³ Other studies indicated it to be composed of (1) the toxin, (2) a nontoxic protein otherwise very similar in properties to the toxin,^{3,15} (3) a dye derived from the bean shells, (4) an allergen, (5) an unidentified substance which tends to keep the toxin in solution at pH 7.0, (6) proteoses,¹⁵ and (7) inorganic salts.¹²

PREPARATION 470 BM 199

About 100 lb of the product designated as 470 BM 199 was prepared¹¹ for field trials at Dugway Proving Ground²² and Suffield Experimental Station, Canada.^{43,44} This ball-milled material was the best available in sizable quantities from the standpoint of high toxicity and small particle size for the field tests held during the spring and summer of 1944.

Ricin-sodium sulfate cake was the starting material for the preparation of 470 BM 199. The cake was ball-milled for 15 minutes in an Abbé porcelain jar mill to yield a product that would pass through a 40-mesh screen and then slurried with 5 parts of its weight of carbon tetrachloride. The sodium sulfate tended to settle to the bottom of the mixture and the ricin concentrated at the surface where it was removed by scooping with a wire screen. This flotation step reduced the salt content of the cake from about 45 per cent to 15–18 per cent. The flotation-purified ricin was suspended in carbon tetrachloride and the slurry ball-milled for 8 hours at room temperature in 1¼ gallon capacity Abbé porcelain jar mill using 5/8-inch steel balls. The product was tray dried at 60 C for 2 hours and then at 82 C for 1½ hours, which gave a white friable cake readily disintegrated by ball-milling for 5 minutes.

Considerable denaturation of protein accompanied the ball-milling operation. The TU values found for different samples of this material ranged from 60 to 100.^{1,9} Examination of a representative sample showed a particle mass median diameter of 6.3 μ , 4.4 per cent moisture, 15.4 per cent ash, and 13.35 per cent nitrogen, of which 64 per cent was soluble and 14 per cent was coagulable.⁹

PRODUCT L703¹¹

A total of about 60 lb of spray-dried air-ground ricin was prepared.¹¹ Lot L703 was examined in the

laboratory for toxicity by inhalation after dispersion as a dust⁹ and similar lots L704 and L826 were tested in the field at the Suffield Experimental Station, Canada.⁴⁶ The small mass median diameters, 3.1 μ for L703 and 3.3 μ for L826,^{29a} are particularly noteworthy.

The starting material for the preparation of spray-dried air-ground ricin was (1) ricin-sodium sulfate cake partially purified by flotation in carbon tetrachloride, and included some (2) ball-milled and (3) hammer-milled products. Preliminary to spray drying, these materials were partially purified by another precipitation with sodium sulfate. The starting material was stirred with 4 parts of water, the pH of the mixture adjusted to 7.0 ± 0.1 , guhr added, and the mixture filtered at 30 C. Sodium sulfate (16.2 per cent of filtrate weight) was added to the filtrate. The resulting slurry was adjusted to a pH of 7.0 ± 0.1 and filtered at 30–35 C. The filter cake was dried at 60 C for 16 hours, ball-milled for 5 minutes to pass a 40-mesh screen, and the sodium sulfate content was reduced by flotation in carbon tetrachloride. Spray-drying 20 per cent aqueous solutions of this flotation-purified ricin gave materials with particle mass median diameters of 6–8 μ .

The spray-dried materials were processed in an air grinder to yield products with TU values averaging 200 and mass median diameters of 2.5–3.5 μ .¹¹ Analysis of lot L703 showed 2.0 per cent moisture, 19.7 per cent ash, 13.2 per cent nitrogen, of which 94 per cent was soluble and 45 per cent coagulable, a TU value of 160, and a mass median diameter of 3.1 μ .^{9,29a}

PREPARATION B1³

Preparation B1 is of interest because of its use for the preparation of toxoid. It was prepared as follows: 7½ g of ricin-sodium sulfate cake, which contained 71 mg of insoluble nitrogen and 650 mg of soluble nitrogen, was suspended in water and centrifuged. The precipitate was washed twice with 30 ml of water, to which was added for the second washing about 0.1 g of sodium sulfate. To the solution and washings (300 ml) was added 175 ml of warm saturated sodium sulfate solution to precipitate the toxin, and the mixture was allowed to stand overnight. The precipitate was centrifuged and reprecipitated twice from a volume of 150 ml with 87.5 ml of warm (37 C) saturated sodium sulfate solution. Additional toxin can be recovered from the filtrates.

B1 is about two-thirds as toxic as the crystalline material. The molecular weight of B1 was determined

to be 85,000 and the isoelectric point to be 5.2. Crystalline ricin and B1 seemed to differ only in toxicity, since by immunochemical, ultracentrifugal, and electrophoretic criteria they appeared to be identical.

12.3 PHYSIOLOGICAL ACTION ^c

Systematic work on the use of ricin as a chemical warfare agent was begun in the United States during the fall of 1942. Its immediate objective was the production on a pilot plant scale of a sufficient quantity of an active product to make possible field trials of methods of dispersal of this novel type of agent. Such toxicological work as was done at this time was directed toward assisting in the control of the plant process and toward the accumulation of basic data on the inhalation toxicities of the product in various species of animal.

When it became evident that the bulk production of a satisfactory material was feasible,^{1,4,11} the question arose of the form in which it should be prepared for dispersal in the field. On the basis of experience in England,^{33,36,37} it was decided that it should be reduced to a finely divided dry powder which could be introduced into munitions either in the dry state or in suspension in an inert volatile liquid. This decision made urgent the need for an extensive investigation of the relation between the particle size distribution in a toxic dust cloud and the inhalation toxicity of the cloud. Thereafter the chief emphasis of all aspects of the program was on this complex problem. It was recognized that the significance of the program did not rest solely upon the potentialities of ricin as an agent for chemical warfare. Ricin was considered, rather, as a readily available prototype of other unstable nonvolatile toxic agents of biological origin which might be exploited as offensive agents by one or other of the warring nations.

The following subsection contains a summary of the available information on the parenteral and inhalation toxicities of standard preparations of ricin. This is followed by a review of the symptoms and pathology of ricin poisoning and a brief discussion of the mechanism of its action.

12.3.1 The Parenteral Toxicity of Ricin

Details of methods of bioassay, of methods of field detection and assessment, and of the relation of particle size to inhalation toxicity will be found in Sec-

tions 12.5 and 12.6 and in Chapter 15, respectively. The summary which follows is concerned only with the toxicities for various species of standard preparations of ricin under laboratory conditions.

Ricin has been stated to be toxic for all vertebrates.⁵ Frogs are sensitive only if kept in a warm environment.⁶⁵ Few invertebrates appear to have been tested. The motility of a ciliate has been found to be arrested by low concentrations of ricin,⁵ but the relation between this effect of a preparation and its toxicity for higher animals has not been established.

The results of the few laboratories that have made comparative assays of a single preparation on a range of animal species are summarized in Tables 1 and 2. In Table 1 they are given as toxicities relative to the toxicity for the rabbit. The high sensitivity of the rabbit is well attested, but there is not full agreement on the order of sensitivities of other species. The majority of the toxicities recorded in the literature have been based upon very few animals and are scarcely more than orders of magnitude. The most extensive series of observations are those made at the University of Chicago Toxicity Laboratory [UCTL],⁹ but even these can be accepted as precise only for the mouse and for the rabbit.

TABLE 1. Relative LD_{50} 's (approximate) of ricin for different species.*

Author Date Reference Route	Osborne 1905 64 Subcu- taneous	Field 1910 56 Intra- muscular	Hunt 1918 24 Subcu- taneous	OSRD 5525 ⁹ 1945 9 Subcu- taneous
Rabbit	1	1	1	1
Rat	..	..	1	1.5
Guinea pig	7	8	5	3
Mouse	..	..	8	8
Sheep	..	..	..	2
Dog	..	7	16	2
Cat	..	2	16	10
Goat	..	30	..	..

* An entry of 10 in this table indicates that for the species in question, ricin was found to be one-tenth as toxic as for the rabbit, etc.

In Table 2 some of the data on which Table 1 was based are given in absolute units. The preparation to which they refer exhibited about 28 per cent of the toxicity of crystalline ricin based on comparative assays on mice. Although the crystalline material is not believed to be molecularly homogeneous, it is definitely the most toxic material which has been prepared in contemporary work. It is suggested, therefore, that the best estimate of the attainable toxicity of ricin is obtained by dividing the LD_{50} for

^c By R. Keith Cannan.

TABLE 2. Estimated LD_{50} ($\mu\text{g/kg}$).

Author	Osborne	Field	OSRD 5525 ⁹ (10-day observation)	
			Standard ricin*	Crystalline ricin (computed)
Rabbit	0.5 (7-day) 100 (2-day)	0.1	10	3
Rat	...	...	15	4
Guinea pig	3.2 (7-day) 100 (2-day)	0.8	30	9
Mouse	...	...	80	24
Sheep	...	...	20	7
Dog	500 (2-day)	0.6	20	7
Cat	100 (2-day)	0.2	100	30
Goat	...	3 (3-day)	...	...

* The OSRD observations were made on the pilot plant product (standard ricin). This had 28 per cent of the toxicity for mice of crystalline ricin.

standard ricin by 3.5. The figures given in the last column of Table 2 have been derived in this manner.

The very high toxicities recorded by Field⁵⁶ for his preparation find no explanation. It is highly improbable that they represent a product many times more toxic than crystalline ricin. On the other hand, his figures and those of Osborne⁶⁵ do suggest that some of the early investigators of ricin succeeded in purifying the toxin to a degree approaching the purity of the crystalline material.

THE RELATION OF THE SURVIVAL TIME TO THE DOSE

The early investigators recognized that the time of survival of animals injected with ricin varied from a few hours to several weeks depending on the dose administered. This relation has been investigated for mice and, less extensively, for rats in several laboratories^{5,9,15,42,61} and has formed the basis of the accepted method of bioassay (Section 12.5). The dose-survival time curves for mice obtained in one laboratory^{9,15} have been found to approximate rectangular hyperbolas which may be represented by the equations

$$\begin{aligned} D(t - 11) &= 430 \text{ (intravenous)} \\ D(t - 13) &= 1,150 \text{ (intraperitoneal)} \\ D(t - 16) &= 2,500 \text{ (subcutaneous)} \end{aligned}$$

where t is the survival time in hours and D is the dose in micrograms of crystalline ricin per kilo bodyweight.

ROUTE OF INJECTION

The above results indicate that the relative toxicities for the mouse by subcutaneous, intraperitoneal, and intravenous injection, respectively, are (for the smaller doses) approximately 1/2.2/6. The subcu-

taneous and intravenous toxicities for the rabbit⁹ are in the ratio of 1/5.

12.3.2 Toxicity by Inhalation

The importance of the particle size distribution of the airborne toxin has been emphasized in the introduction. In one extensive investigation of this problem,⁹ two methods of varying the particle size were used. In one, animals were exposed to atomized aqueous solutions of ricin containing varying amounts of glycerol. The mean particle size in the aerosol varied with the amount of nonvolatile solvent in the solution. The other type of experiment was the exposure of animals to dust clouds generated from powdered standard ricin which had been reduced to varying degrees of fineness by milling or spray drying. The results are summarized in Table 3.

TABLE 3

A. Inhalation toxicities of atomized solutions of standard ricin.

	$L(Ct)_{50}$ (mg/min/m ³)		
MMD (μ)	1.4	4.6	6.6
Rabbit	4	8	10
Guinea pig	7	15	..
Mouse	9	40	45
Dog	24	45	..
Cat	24	50	..
Rat	50	120	..
Monkey	100	...	..

B. Inhalation toxicities of dry dusts of standard ricin.

Preparation	Ball-milled		Spray-dried		Atomized solution
MMD (μ)	10	6.3	5.9	3.1	1.4
<i>Relative toxicities</i>					
Mice	3.5	2.8	0.5	6.5	100
Rabbits	5.7	5.3	..	30	100
% mass below 3 μ	7.5	10.0	3.0	45	100?
% mass below 2 μ	3.0	3.2	0.6	8	100?

It would appear that the toxicity increased as the mass median diameter [MMD] of the cloud diminished. Indeed, there is some justification for the conclusion, in the cases of mice and rabbits, that the toxicity was roughly proportional to the fraction of the airborne mass which was present in particle sizes smaller than 2-3 μ in diameter. The reader is reminded that the MMD is an inadequate description of the characteristics of a dust cloud in which the particles differ in shape and density as well as in size and is referred to Chapter 15 for a discussion of the

relation of these factors to the probability that an inhaled particle will penetrate the nasal barrier.

Although the most toxic aerosol was that with the MMD of $1.4\ \mu$ it is improbable that this represents the maximum attainable inhalation toxicity. Some allowance for nasal retention and for incomplete retention in the lungs should probably be made. Even so, the inhaled doses of the $1.4\text{-}\mu$ aerosol for mice and rabbits, which may be computed from the minute volumes of respiration and the $L(Ct)_{50}$'s, are approximately equal to the LD_{50} 's by intravenous injection.⁹ That is to say, ricin is at least as toxic by inhalation as by vein. That it is probably more toxic in the lungs is indicated by the fact that the approximate LD_{50} , when solutions were injected directly into the trachea of rabbits, was $0.5\ \mu\text{g}/\text{kilo}$. In cats, dogs, and rats it was about $5\ \mu\text{g}/\text{kilo}$.⁹ In contrast with these results were the very low toxicities resulting from the nasal instillation of ricin.⁹

When solutions of ricin are instilled in the eyes of animals in sufficient amount, enough may be absorbed to be lethal.²⁴ Only small amounts are necessary to produce serious local injury. The instillation of $1.5\ \mu\text{g}$ of crystalline ricin produced corneal damage in a rabbit's eye which disappeared in 10–14 days.⁹ A particle of $100\ \mu$ in diameter ($0.5\ \mu\text{g}$) implanted in the eye resulted in a conjunctival reaction persisting for a week. Corresponding lesions in the eyes of rats and guinea pigs required five to ten times this dose. It must be remembered that only large particles will impinge in the eye from a cloud and that such particles will tend to precipitate rapidly under wind conditions favorable for the persistence of a fine particulate cloud. Clouds of fine dusts such as are highly toxic by inhalation would therefore be unlikely to contain a concentration of coarse particles which would present a serious hazard to the eyes.

12.3.3 The Toxicity of Ricin for Man

The ingestion of two castor beans has been fatal in man.^{24,54,68} It has been estimated that this corresponds with a lethal dose of about $0.3\ \text{mg}$ of purified ricin per kilo. It has been suggested that ricin is about 100 times as effective by vein as by mouth.⁶⁰ On this basis the intravenous lethal dose for man would be as small as that for the rabbit. Such computations are highly precarious, but other evidence has been advanced to indicate that man is quite susceptible to ricin poisoning.^{33,41}

Elsewhere in this section are described symptoms of mild poisoning in a number of individuals who had

probably been exposed to low concentrations of airborne ricin. It is significant however that no serious casualty has occurred in the pilot plant, in the explosion pit at Dugway Proving Ground, or in laboratories studying the dispersal of ricin. The atmospheres in all these places must have been contaminated with ricin dust.

It has also been suggested that the handling of solutions of ricin presents a skin hazard,⁴ but the opinion of most investigators who have long worked with such solutions is that the hazard is small if elementary cleansing precautions are taken.

12.3.4 Symptoms of Intoxication

Laboratory animals show no evidence of intoxication for several hours after the injection of a dose which will kill them in 24 hours. Thereafter their fur becomes ruffled, they grow restless, and refuse food. As the time of death approaches, diarrhea is frequent, breathing becomes dyspneic, their bodies feel cold to the touch, and their eyes may become sealed with exudate. Finally, the animals become moribund and die in coma or, more frequently, after a series of violent convulsions. With smaller doses the sequence of events is similar, but their time course as well as the initial latent period are more protracted.

Some 150 cases of poisoning in man have been reviewed.^{24,54,68} Most of these have been the result of the accidental eating of castor beans. In some cases weakness and prostration were the only symptoms. In more serious attacks, there was nausea and vomiting, epigastric pain, cramps in the limbs, a weak pulse, and a rapid respiration with a rise in body temperature. Fatal cases passed into collapse followed by convulsions. Symptoms might be delayed for 2 to 14 days, or, surprisingly, might be evinced within 1 hour after ingesting the beans.

Among the personnel working with ricin in the United States throughout 1943–45, there were no serious cases of poisoning, although there were a number of minor illnesses attributable to exposure. These were probably the result of inhaling airborne toxic dust. Two types of reactions among laboratory workers have been distinguished.⁹ One — the immediate reaction — resembles that of an individual sensitized to a foreign protein. The symptoms have varied from a protracted bout of sneezing to a severe asthmatic attack with violent coughing and retching. The symptoms disappeared within an hour. The second type of reaction probably corresponds to the toxic effect in animals. Symptoms were delayed for

4 to 8 hours. There was then a sharp febrile response, tightness of the chest, tracheitis, aching joints, nausea, dyspnea, and coughing. Some hours later the onset of profuse sweating was commonly the signal of the alleviation of most of the symptoms.

Somewhat similar observations have been made by the British, who have obtained local and general reactions by the intradermal injection of very small doses of ricin preparations³³ (see Section 12.4.3).

12.3.5 Pathology

Accompanying the outward signs of intoxication in animals has been noted an early fall in body temperature,⁵² which may be preceded by a rise.⁵⁷ In rabbits, it has been reported that the blood pressure falls from 100 to 65 mm of mercury at an early stage and remains at this level until death.⁷ There appear to be no notable changes in the blood picture.²⁷ It is generally agreed^{9,10,47,52} that about 20 hours after the injection of an LD_{50} dose there is a leucocytosis, with a simultaneous increase in both lymphocytes and polymorphonuclear leucocytes.¹⁰ A transient fall in red cell count has been recorded,^{9,10} but others report no change in red cell count, in red cell volume, or in sedimentation rate.⁵² Within 20 hours after an LD_{50} dose the clotting time was found to increase to three times its normal value and remain at this level till death several days later.⁷ An extreme terminal hypoglycemia⁷ and acidemia⁵² have been observed in rabbits and in rats and a rise in blood phosphatase has been reported.⁵²

Careful reports of the gross and microscopic pathology of animals dying after the parenteral administration of ricin are found in the early literature.⁵⁵⁻⁵⁷ This information is reviewed and extended in Chemical Warfare Service Monograph 37, written in 1918.²⁴ Between this time and 1940, students of ricin became preoccupied with the chemical and immunological characterization of the toxin and with the hemagglutinating activities associated with it. Little was added to our knowledge of the physiological action of the toxin. During World War II, extensive pathological examinations of animals poisoned with ricin were made in England,³⁹ in the United States,^{9,10,22} and in Canada.⁴⁷ Some of these were confined to post-mortem examination of animals killed by the injection or inhalation of the toxin,^{9,22,39} whereas others relate to animals sacrificed at chosen times after the parenteral administration of lethal or sublethal doses.^{10,58} Bearing these differences in procedure in mind, it may be said that there is substan-

tial agreement between the laboratories referred to and the early reports in the open literature.^{55-57,65} It is possible, therefore, to summarize the situation in the following general conclusions.

PARENTERAL ADMINISTRATION

1. There is mild to moderate congestion and edema of the lungs.
2. There is mild degeneration of the intestinal epithelium at supralethal doses only.
3. There is necrosis of the liver at and below LD_{50} doses.
4. There is hyperplasia of the spleen at sublethal doses and involution at higher dosage.
5. There is fragmentation and involution of the thymus at all doses.
6. There is congestion and delayed necrosis of the adrenal in rats but not in rabbits.

The occurrence of pin-point hemorrhages throughout the body has been emphasized by some^{47,57} but minimized by others. Less consistent findings have been necrobiosis of reticuloendothelial cells and bone marrow, cloudy swelling of the kidneys, and fatty degeneration of heart muscle. No differences between the effects of crystalline ricin and of amorphous preparations have been observed¹⁰ nor have any striking differences in the responses of different species been observed.⁹

INHALATION

The pathology is almost entirely confined to the thorax.^{9,22} The lungs are dark and greatly increased in weight and are filled with edema fluid. The abdominal organs are normal except for some fatty degeneration and, occasionally, hyaline infiltration and necrosis of the liver.

INGESTION

The effects of ingesting the toxin have been investigated in fatal cases of poisoning in man.^{24,54,68} The chief post-mortem findings have been extreme congestion of the stomach and intestines.

12.3.6 The Mechanism of Action of Ricin

Such pathological work as was carried out in the United States in 1943-45 was incidental to the program outlined in the introduction. No systematic investigation of the mechanism of action of ricin was undertaken and our knowledge of this subject remains fragmentary. We are, indeed, as ignorant of the nature of the action of ricin as we are of the actions of those bacterial toxins which exhibit a simi-

lar delayed effect and ill-defined pathology. Apart from revealing local effects depending upon the route of administration, pathological reports betray no characteristic lesions which would indicate the intrinsic nature of the toxic action.

The death of animals in convulsions is probably the result of hypoglycemia. It has been found that the blood sugar of rabbits and rats remains normal until a few hours before death, when it falls precipitously to convulsive levels.^{7,52} The toxic action, however, is not primarily a reversible disturbance in carbohydrate metabolism. The liver glycogen is found to be very low at death, but it has not been possible to induce glycogen storage in poisoned animals by injecting glucose to maintain a normal blood sugar level. Nor has life been prolonged by this means.⁷

One of the earliest theories of the action of ricin was that it was an enzyme. This was thought to explain its great potency. It was also thought that its delayed action might plausibly be attributed to the time required for the enzyme to build up a lethal concentration of the hypothetical product of its activity. In this connection it should be borne in mind that several enzyme activities — phosphatase, lipase, esterase — are exhibited by extracts of castor beans. Purification of the toxin is not, however, accompanied by enhanced enzyme activity. Indeed, it has been stated that crystalline ricin is free from phosphatase and lipase action.^{12,13} Recently a Canadian laboratory has reported that ricin preparations hydrolyze adenosine triphosphate (ATP).^{43,50} They further observed that ricin inhibited the beat of the isolated frog's heart and that the beat was restored by the addition of ATP. This would suggest that ricin may act by interfering in those basic metabolic reactions whereby the energy of metabolism is conveyed to the functioning structures of tissues. Data are, however, not yet available to indicate whether the concentration of crystalline ricin which is required for effective adenosine triphosphatase action is such as to make plausible the hypothesis that its lethal action is dependent on this property. Moreover, in one investigation⁷ the action on the frog's heart was not confirmed. No increase in nucleotide in the blood of animals poisoned with ricin was observed. Ricin did not cause a hydrolysis of ATP in the blood of dosed rats.⁵²

It may be submitted that it is just as plausible to attribute a disturbance in metabolism to the blocking or distortion by the toxin of the action of an enzyme

native to the cells of the animal as to consider it to be the result of the invasion of those cells by a foreign enzyme in the form of the toxin.

An early theory of the action of ricin was based on the hemagglutinating properties of ricin preparation.^{24,28} If this action were manifested *in vivo* profound disturbances in circulation might be responsible for the toxic effect. Unfortunately the concentrations of ricin required to agglutinate red cells *in vitro* are greater than those established in body fluids by lethal doses of ricin. Moreover, the agglutination of red cells is inhibited by serum^{3,19c} and crystalline ricin is very much less potent as an agglutinin than are cruder preparations.^{3,15} Finally, the absence of thrombotic lesions would seem to deny the theory. Although the hypothesis has little to support it, it should be recorded that tissue cells as well as erythrocytes have been shown to be agglutinated by crude ricin and, in the case of the tissue cells, the action is accentuated rather than inhibited by addition of serum.²⁴

One investigator⁵² has drawn attention to the similarity between intoxication by ricin and circulatory shock. He has found some evidence of diminished blood volume in poisoned rats and of reduced peripheral circulation in the rabbit. The latter effect he was inclined to attribute to pooling of blood in the splanchnic area. He considered, but dismissed, the thought that this condition might be due to capillary blockage resulting from agglutination *in vivo*. An incidental observation bearing on this question was that the rate of absorption of iron from the gut and the amounts deposited in tissues were increased in poisoned animals. He draws attention to a similar observation on animals in peptone shock.⁶⁹

In conclusion it is worthy of remark that no effect of ricin on unicellular organisms or isolated tissues has been clearly established. Much more work in this field is desirable as are more detailed studies of the time course of metabolic disturbances in poisoned animals and the level of differentiation of tissue organization and function at which susceptibility to poisoning first becomes manifested.

12.4

IMMUNOLOGY^d

In the United States active research on the immunology of ricin was initiated in February 1943 by NDRC Division 9 (Section 9.4.2, Immunochemical

^d By Birdsey Renshaw.

Studies).^{3,18} Related work was subsequently taken up by other NDRC investigators,^{9,14,15,17} by the Chemical Warfare Service,^{22,23,26,27,28,31} and by the Committee for Medical Research.¹⁹ At the time the NDRC research began there were available, in addition to the open literature on ricin, an account of studies carried out for the Chemical Warfare Service during World War I²⁴ and reports on preliminary work conducted in the United Kingdom during 1940, 1941, and 1942.^{33,36,40,41} More recently Canadian investigators have made a significant contribution.⁵³

The principal objective was to provide and evaluate immunological procedures for protection against and treatment of ricin poisoning. With respect to protection, the aim — not yet attained — was the production of a toxoid which could practically be used to immunize troops. With respect to treatment, the problem — now satisfactorily solved — was the production and evaluation of potent antiricin serums and antibody globulin preparations. A secondary objective was the study and evaluation of immunological methods for detection and estimation. By-products of the immunochemical work have been significant contributions to the purification and physicochemical characterization of ricin.^{3,18}

For purposes of orientation it may be stated at the outset that immunological, ultracentrifugal, and electrophoretic studies on ricin preparations from castor beans of different source and color have failed to reveal the existence of more than one heat-labile, toxic antigenic protein.^{3,18} On the other hand, a non-crystalline fraction (B1) from castor beans, which by these criteria is identical with crystalline ricin, is not so toxic as the latter.^{3,19c} Furthermore, solubility studies do not reveal the crystals to be homogeneous.² It is also known that castor beans contain, in addition to heat-labile toxic protein, one or more heat-stable antigenic substances of low molecular weight (allergen); small amounts of allergen appear to be present even in crystalline ricin.^{14,53}

12.4.1 Preparation of Ricin Toxoids

Incomplete success has attended efforts to produce from ricin a toxoid possessing high antigenic potency coupled with negligible toxicity and skin-necrotizing properties. The available toxoids are satisfactory for eliciting vigorous antiricin production in animals. The best has been recommended for the active immunization of volunteers on an experimental scale but is not considered suitable for practical use in the routine immunization of troops.

The most satisfactory toxoid has been prepared by formalinization of the toxin as follows: ^{3,18m} ricin at a concentration of 0.5 mg ricin nitrogen per milliliter in 0.15M sodium chloride plus 0.02M phosphate buffer at pH 7.4 is treated with 5 per cent formalin for 5 days at 37 C. Originally, partially purified pilot plant ricin (B1 fraction ^{3,18c,e,i,m,q,r,s,t,u}) was used. Recently crystalline ricin has been utilized with similar results ^{19a} and will undoubtedly be employed in all future work. For best results the toxoid is precipitated with alum or protamine. The resulting toxoid is about one-thousandth as toxic for mice as native ricin.^{3,18m,t} However, subcutaneous injection of as little as 0.1 µg of the toxoid nitrogen produces skin necrosis in some rabbits,^{19a} and in the form of an aerosol the toxoid is only about 15 times less potent than native ricin as a lung injurant.^{19a}

Some observers believe that formalinization in a more alkaline medium yields a better toxoid.¹⁴ Undoubtedly a greater diminution of toxicity is effected under these conditions, but the indications are that antigenicity is more than correspondingly reduced.^{3,18r,t,u} Precise evaluations of toxoids prepared at pH values differing by only 0.1–0.2 unit are not available.²¹ The concentration of formalin is not critical; even high concentrations do not effect complete detoxification, and 0.5 per cent suffices to produce a toxoid suitable for many purposes.³ Some consideration has been given to the chemical reactions that occur during toxoid formation.^{14,15}

No success has attended numerous attempts to produce a toxoid more effective than that just described. Among the procedures to which ricin has been subjected are the following: oxidation with chlorine or permanganate;⁴⁰ ultraviolet irradiation at low intensities ^{3,18e,f} and for short times at high intensities;^{3,18u} acetylation;^{3,18i,k} tryptic digestion;^{19a,c} peptic digestion;^{19b,c} treatment with ninhydrin;¹⁴ and heating.¹⁴ A toxoid prepared by shaking ricin with toluene showed some promise in preliminary tests but remains to be completely evaluated.^{19b} Injections of formalinized toxoid treated with normal serum and of specific precipitates of formalinized toxoid with antiricin rabbit serum proved unsatisfactory for active immunization.^{3,18r} A few additional procedures have been suggested²¹ but were not evaluated before the work terminated.

A finding of significance is that a purified but non-crystalline fraction (B1) prepared from pilot plant ricin is immunologically identical with crystalline ricin but possesses only 60 per cent of the toxicity of

the latter.^{3,17b,e,18s} This observation suggested (1) that some form of detoxified ricin either exists in castor beans or is produced in the process of extraction and purification, and (2) that crystallization effects at least a partial separation of the toxic from the detoxified material. That detoxified material immunologically indistinguishable from ricin is indeed present in castor beans is suggested by the further finding that crude aqueous extracts from the beans also possess considerably more immunologically active material per unit amount of toxic material than does crystalline ricin.^{3,18u,19a,c} Up to now it has been possible to effect only a very incomplete separation of the toxic and nontoxic fractions.^{19a,c} However, further study of the conditions and factors responsible for the origin of detoxified ricin in castor beans might lead to a solution of the toxoid problem.^{19c} In such work the changes which may take place in developing and germinating beans should be examined.

There is evidence that castor bean allergen^{66,67} is not completely removed from the heat-labile ricin by crystallization⁵³ or even by repeated recrystallizations.¹⁴ Injections of a toxoid containing even small amounts of allergen conceivably might render men hypersensitive to the allergen contained in subsequently injected toxoid, and to sublethal dosages of airborne ricin containing allergen. Some workers are inclined to minimize the practical importance of this possibility; to others¹⁴ it has been a source of great concern. Animal experiments bearing on the point are reviewed in Section 12.4.4, and limited human data are presented in Section 12.4.3.

12.4.2 Antiricin

Potent antiricin rabbit, horse, and goat serums have been obtained by a series of injections, first subcutaneous and subsequently intravenous, of ricin toxoids.^{3,18,28} Immunization can be continued with alum-precipitated but otherwise untreated ricin. For therapeutic purposes the hyperimmune serums may be used as such, but the antibodies are preferably purified to lessen the likelihood of immediate reactions and serum sickness.

STANDARDIZATION OF ANTISERUMS

During World War II antiricin has been estimated with reference to an American Standard Antiserum arbitrarily assigned a potency of 100 units/ml.^{20,31f} Each milliliter of this serum^e contains antibody

^e Available at the Medical Research Laboratory, Edgewood Arsenal.

equivalent to about 7,500 mouse LD_{50} doses of ricin; that is, by the toxicity test described below it neutralizes 200 μ g of crystalline ricin nitrogen¹⁸¹ or 500 μ g of nitrogen of the relatively impure pilot plant preparation against which it was first tested.^{31f}

Two tests have been developed for the quantitative assay of antiricin titer:^{3,18c}

1. *Toxicity-neutralization.* Solutions of known amounts of ricin and of antiserum are mixed in 0.9 per cent saline, incubated at 37 C for $\frac{1}{2}$ hour, and injected intraperitoneally into mice. The minimum volume of serum in the mixture for which mice survive for 10 days is considered to be equivalent to the amount of ricin used. The toxicity-neutralization test may be used to detect as little as 0.2 unit of antibody and is the method of choice if time permits.

2. *Inhibition of hemagglutination.* Portions of ricin (e.g., 2 μ g in saline) are mixed with decreasing volumes of serum and saline is added to a volume of 0.8 ml. After incubation at 37 C for $\frac{1}{2}$ hour, 0.2 ml of a 4 per cent suspension of washed human erythrocytes of blood group O are added. The extent of agglutination is recorded after shaking and incubating at 37 C for 1 hour. The minimum amount of serum which completely inhibits hemagglutination is considered equivalent to the amount of ricin used. Because of nonspecific inhibition by normal serum,^{18d} this test cannot be used to measure less than 5 units of antibody per milliliter.^{18c,d} In the choice of ricin for use in this test consideration must be given to the fact that the hemagglutinating properties can be reversibly masked under some circumstances.^{3,15,18m,31g}

POTENCY OF ANTISERUMS

The use of graded series of injections of ricin toxoid and/or native ricin has yielded in rabbits antiserums having potencies as high as 250 units/ml.³ In horses serums possessing 150 units of antibody per milliliter have been obtained.³ However, few animals have been observed with circulating antibody titers greater than that of the standard, and most animals in any series will attain titers more or less below it. Nevertheless, the pooled serum from a group of adequately immunized rabbits possesses what can be considered for therapeutic purposes a high and effective titer.

PURIFICATION OF ANTIRICIN

To reduce the possibility of reactions from the therapeutic use of antiricin serum, methods which had been used for the partial purification of other antibodies were applied.³ Sufficient experience has

been gained to make possible the production of concentrated, partially purified horse or rabbit antiricin rapidly and on as large a scale as any program might require.

Antiricin globulin from immunized rabbits was obtained in almost quantitative yield by 45 per cent saturation of the diluted serums with sodium sulfate at 37 C. The precipitate was dissolved in water, merthiolate added as a preservative, and the solution sterilized by passage through a Chamberland filter.^{18g,1} The ampouled material possessed an antiricin potency of 50 to 125 units/ml and was prepared in sufficient quantity for distribution to the Chemical Warfare Service and NDRC laboratories engaged in work on ricin. Prompt intravenous injection of 25 ml was recommended in the event of accidental inhalation of ricin aerosols.

Horse antiricin was partially purified by isolation of the pseudoglobulin fraction^{18m,31g,h} or by peptic digestion by the Parfentiev method.^{18q,31h} The latter method was used to process 16 l of horse plasma assaying 50 units of antiricin per milliliter. The yield was 1,090 ml of purified, modified globulin solution assaying 500 units/ml.^f

THERAPY WITH ANTIRICIN

Antiserum or purified antibody globulin is of considerable therapeutic value if promptly administered. Its effectiveness rapidly decreases with increase in the time between poisoning and therapeusis, and no benefit is obtained after the delayed symptoms of poisoning have appeared.^{3,18,31,40}

Serotherapy is effective against injections of at least several lethal doses of ricin if sufficient antiricin is administered promptly.^{3,18,31} Typical data for mice are presented in Table 4.^{18j}

After exposure to airborne ricin the pathological effects occur mainly in the lungs³¹ⁱ and the animals are not completely protected against pulmonary injury even by immediate therapy with injected antiserum.^{31d,f,j} However, the use of antiserum has definite life-saving value up to 6 hours after gassing and is perhaps of limited benefit even as late as 10 hours.^{3,18l,n,31d} Illustrative data are presented in Table 5.¹⁸ⁿ The data reveal the desirability of utilizing a large amount of antiricin. Equivalent amounts of rabbit antiserum, purified rabbit antibody globulin, and purified horse antibody pseudoglobulin ap-

^f This material, put up in ampoules each containing 12 ml, is available at the Medical Research Laboratory, Edgewood Arsenal.

TABLE 4. Therapeutic use of antiricin in mice poisoned with ricin by intraperitoneal injection.^{18j}

Mice were injected intraperitoneally with about 20 lethal doses (2 μ g of B1 ricin nitrogen) and subsequently injected intraperitoneally with ten times the neutralizing equivalent of antiricin (rabbit antiserum).

Treatment	Mortality			Total
	0-24 hr	24-48 hr	2-10 days	
No serum	36	0	0	36/36
Serum 0.5 hr after ricin	0	0	0	0/37
Serum 2 hr after ricin	1	0	5	6/38
Serum 5 hr after ricin	9	3	9	21/37

TABLE 5. Therapeutic use of antiricin in mice poisoned with ricin by inhalation.¹⁸ⁿ

Mice were exposed for 10 minutes to an aqueous aerosol of ricin at a nominal concentration (about 8 μ g B1 ricin nitrogen per liter) which was equivalent to at least ten times the LC_{50} . Either 10 or 100 units of antiricin in the form of purified rabbit antibody globulin were administered intraperitoneally at various times after the exposure.

Antiricin administered	Time of treatment	Mortality			Total
		0-4 days	4-8 days	8-10 days	
100 units	None	20	0	0	20/20
	1 hr	0	0	1	1/17
	4 hr	2	3	0	5/19
	10 hr	5	9	2	16/18
	24 hr	17	3	0	20/20
10 units	None	16	0	0	16/16
	1 hr	2	4	1	7/20
	4 hr	1	10	0	11/18
	10 hr	11	7	2	20/20

pear to possess approximately the same therapeutic efficacy.^{3,18l,n}

PASSIVE IMMUNITY

Passive immunity results from the injection of high-titer antiserum or partially purified antiricin.^{3,18,31a,b,c,e,f} On the basis of these animal experiments, however, the protection cannot be expected to persist for more than 1 or 2 weeks at most. Thus passive immunization would have limited usefulness as a practical method for protecting troops.

12.4.3 Active Immunization against Ricin

Injection or inhalation of native ricin in small doses evokes antiricin formation^{31a,c} and immunity^{27,40} in surviving animals. The response is sometimes striking, particularly after repeated administration of the toxin. Practically speaking, however, active immunization must be attained by the use of a toxoid.

TABLE 6. Resistance to airborne ricin of rabbits immunized with six injections of formalinized ricin.^{19c}

The exposures in the gassing chamber were of 10 minutes' duration. The 10-minute LC_{50} for nonimmunized rabbits was about 0.5 μg ricin nitrogen per liter. Thus the exposures were to approximately 4 and 20 times the $L(Ct)_{50}$ dosage.

Interval between last toxoid injection and exposure	Serum antibody titer before exposure (units)	Conc. of ricin in gassing chamber (μg nitrogen/l)	Deaths in 10 days	Lung pathology in survivors 14 days after exposure	Serum antibody titer in survivors 14 days after exposure
12 days	0.8–3.3	2.3	0/10	– to +++	1–8
	1.0–3.3	11.8	3/9	+ to +++	6.5–12
3 months	<0.2–0.8	2.3	0/7	– to +++	8–>30
	<0.2–0.2	9.8	4/7	++ to +++	12–>30
5 $\frac{2}{3}$ months	<0.2–0.6	2.1	3/8	– to ++	2–4
	<0.2–0.2	11.0	5/7	+ to +++	8–>30

STUDIES WITH ANIMALS

Several injection schedules have been used for studies with rabbits on the development of active immunity to inhaled ricin.^{3,19c} The first schedule consisted of three subcutaneous injections at 5-day intervals of formalinized toxoid in the amounts of 2.5, 5, and 10 μg of ricin nitrogen per animal, respectively. This schedule resulted in circulating antibody levels of 1–3 units per milliliter and many animals survived exposure to about 20 $L(Ct)_{50}$ dosages of airborne ricin 10 days after the last injection. However, the injections of toxoid produced severe skin reactions with necrosis. A schedule of six injections conferred equal or greater immunity and the severity of the local reactions at the sites of injection was greatly reduced, although necrosis was not absent in all instances.^{18q,19a,c} A dosage sequence of 0.1, 0.2, 0.5, 2, 10, and 20 μg of toxoid nitrogen is believed preferable to a schedule composed of doses of 0.1, 0.5, 2, 5, 10, and 20 μg .

Some of the characteristics of the antibody response and protection effected in rabbits by six toxoid injections are illustrated by the data of Table 6.^{19c} Maximum antibody response and protection is attained 10 to 20 days after the last injection. At this time the circulating antibody levels are 1 to 3 units per milliliter of serum. The animals are immune to the lethal effects of at least several $L(Ct)_{50}$ dosages of airborne ricin, but the development of lung lesions is not prevented. Circulating antibody titer then falls progressively to reach levels of the order of 0.2 unit per ml after 2 to 4 months.^{3,18q,19c,31c} In spite of the low level of circulating antiricin, some resistance to airborne ricin persists.^{3,18q,t,19c}

After circulating antiricin has reached a low level, an injection of toxoid produces only a moderate in-

crease in circulating antiricin.^{3,18r,u} In contrast, a very striking increase in circulating antiricin, to 5–30 units per ml of serum, is produced by a single exposure to a sublethal dosage of airborne ricin.^{3,18s,u,19c} The effect is to be seen when the exposure follows by only 12 days the last of a series of toxoid injections. It appears to be more marked after longer times, when the circulating antiricin evoked by the toxoid injections has fallen to low values.^{19c} A second exposure to airborne ricin does not elicit a pronounced further increase in the circulating antibodies.^{18u} These findings suggested that, for purposes of active immunization, controlled exposure to aerosols of ricin *toxoid* might effectively reinforce the effects of toxoid injections. No studies on immunization by inhalation of toxoid have as yet been made, however, except for one experiment in which previously untreated rabbits were given a single exposure to airborne formalinized toxoid.^{19a} Twelve days later none of the survivors had developed a circulating antiricin titer as great as 0.2 unit per ml. Challenge exposures to airborne ricin were not made.

Actively sensitized guinea pigs possess considerable immunity against the toxic effects of ricin.^{3,18p,t} Subsequent to exposure to 15–40 $L(Ct)_{50}$ dosages of airborne ricin, a high proportion of the animals (i.e., 31 of 33) that recovered from the initial anaphylactic reaction survived indefinitely. This degree of resistance was present in the three animals tested as late as 116 to 173 days after sensitization.

Although the results show that considerable resistance to inhaled ricin can be achieved by immunization with formalinized toxoid, it has been emphasized that the resistance has been measured by a statistical increase in the number of animals surviving challenge exposures and that the surviving ex-

posed animals usually develop lung lesions.^{19c} Some of these lesions are severe and apparently predispose the animals to bronchopneumonia.

HUMAN IMMUNIZATION

With regard to immunization of large numbers of troops, representatives of the Surgeon General (Army) have indicated that practical considerations make it highly desirable to limit the number of injections of toxoid to one or at most two or three spaced over a period of 4 to 6 weeks.²⁰ The animal experiments give no reason to believe that effective immunization can be obtained with so few injections of currently available toxoids at doses sufficiently small to preclude very severe local reactions. There is, moreover, no evidence that effective immunity, if once produced, would persist at high levels for more than a few months.

No experiments on human immunization have been made. However, the use of formalinized toxoid at a dosage schedule similar to that employed for immunizing rabbits has been recommended as safe for test with volunteers. It was felt that data on the local effects produced and on the levels of circulating antiricin attained would help to orient the further course of the work.

Numerous serum samples from men working with ricin have been assayed for circulating antiricin by the toxicity-neutralization test.^{3,18d,e,n,u} No significant amounts of circulating antibody were found before exposure to ricin. Considerable antiricin (i.e., up to 2.5 units/ml) was found in the serums of men who had handled ricin at the pilot plant for several months or more. The highest levels were found in men having histories of either cuts and abrasions or symptoms traceable to ricin. There is no direct evidence as to the degree of immunity possessed by these individuals.

In the experience of the University of Chicago Toxicity Laboratory two types of reaction to accidental exposure to ricin have been observed.^{9,17b} One, a delayed reaction, sets in after a latent period of 5 to 8 hours. A febrile response then occurs, accompanied by tightness of the chest, tracheitis, aching joints, nausea, dyspnea, and coughing. In 8 to 12 hours the onset of profuse sweating has been accompanied by alleviation of all symptoms except the cough and tracheitis, which sometimes persisted for several days. This reaction appears to correspond with the toxic reaction in animals. The second type of reaction is immediate and resembles that of sensi-

tization. In mild cases violent sneezing of several minutes' duration starts within a minute after exposure. In more severe exposures there have been asthmatic difficulties of breathing with violent coughing and retching; these symptoms disappear within an hour, leaving only a mild cough and a slight fever. Subjects who responded to exposure with an immediate reaction did not show the delayed symptoms. All had worked with ricin over a prolonged period of time. Little or no antibody was found in serum samples taken from four individuals following reactions of the immediate type.^{3,18u}

Somewhat similar observations were made earlier by the British and give some basis for interpreting the two classes of responses.^{33,40,41} It was found that some men working with ricin accidentally acquired immunity. Their serums contained antiricin, and the intradermal injection of ricin produced less than the usual amount of local damage. Other men became hypersensitive. Their serums contained no detectable antiricin. Intradermal injection of 1 μ g of toxoid produced immediate redness and swelling, in marked contrast to the usual effects of toxin which take many hours to develop. Moreover, the substance causing the immediate cutaneous response was not ricin, for it survived treatment with bleach or with heat sufficient to destroy the toxin. It would seem that the immediate responses which in men sometimes follow inhalation or injection of ricin preparations may be associated with hypersensitivity to castor bean allergen, whereas the delayed responses are, as indicated above, due to the toxic effects of ricin itself.

A few British observations serve to emphasize the high sensitivity of men to the toxic effects of ricin.⁴⁰ Intradermal injections of 0.3 μ g of a preparation which may have been about one-fifth as toxic for mice as crystalline ricin⁹ produced a large area of inflamed swelling with some local necrosis. Similar injections of 1.3 μ g produced more marked local effects and in addition pyrexia, leucocytosis, and malaise sufficient to keep the men off duty for several days.

12.4.4 Immunological Methods for Detection and Analysis

The principal immunological methods for detection of ricin depend upon (1) the agglutination of red blood corpuscles by the toxin, (2) the precipitin reaction between ricin and antiricin, and (3) the anaphylactic responses of sensitized animals. The rela-

tive merits and limitations of these tests have been evaluated.^{3,18}

HEMAGGLUTINATION

The agglutination of human red cells by ricin was used as a laboratory test for the toxin during World War I.²⁴ It has also been studied and utilized in field tests during World War II.^{3,15,18b,1,m,22,23,26,28} It is simple, rapid, and sensitive^g to about 0.3 μg of ricin nitrogen.¹⁸¹ In the form of a test developed during field trials it detected 10 μg of ricin nitrogen in 1 minute, 4 μg in 2 minutes, and 2 μg in 5 minutes.²³ Its specificity and sensitivity do not, however, match those of the other immunological methods, and as an analytical procedure it is at best only semiquantitative.^{3,18m} It must be viewed with suspicion in the case of detection and analysis of unknown ricin samples because of the possibility that hemagglutinating properties can be masked.^{3,15,18m,31g}

PRECIPITIN REACTION

Although the addition of ricin to normal serum produces a precipitate under certain conditions, much smaller amounts suffice to produce a specific precipitate with antiricin serum. Thus the precipitin reaction is highly specific and sensitive; it is capable of detecting 1 μg of ricin nitrogen within 5 minutes, and much smaller amounts in longer times.^{3,18h,1} Although less subject than hemagglutination to extraneous conditions, it must be borne in mind that serum protein is precipitated by the ions of heavy metals which are present in smokes of various kinds.³ With the limitation that different ricin preparations possess different ratios of toxic potency to immunological activity (Section 12.4.1), the quantitative precipitin test affords a very accurate method for the estimation of ricin.^{3,18p,r,s} Under optimal conditions it is accurate to about 1 per cent.

ANAPHYLACTIC RESPONSES OF SENSITIZED ANIMALS

The anaphylactic response of actively sensitized guinea pigs appears to provide the most rapid, specific, and sensitive method for the detection of airborne ricin or ricin dusts that have settled on surfaces.^{3,22} The animals must be watched, however, and the possibility of desensitization guarded against.^{3,18n} Passive sensitization was earlier employed by British investigators^{33,40,41} but active immunization has the advantages of inducing much more prolonged sensitization and, probably, greater

sensitivity.^{3,18i,k,n,p,t} The rapidity and sensitivity of the reactions was demonstrated by tests in which characteristic responses were evoked within 1–3 minutes after exposure to ricin dust at the lowest nominal concentrations tested, 0.03 μg ricin nitrogen per liter.^{3,17a,18n} This concentration was in the order of one-thousandth the 10-minute LC_{50} for mice.

Anaphylactic reactions of guinea pigs are known to be highly specific. In the case of the studies with ricin, however, there has been debate, not yet resolved, as to whether the reactions are due to sensitivity to the toxin itself or to contaminating castor bean allergen. The evidence that guinea pigs can be sensitized to ricin itself, irrespective of the possibility that hypersensitivity to allergen may also occur, may be summarized as follows:

1. Guinea pigs passively sensitized by intravenous injections of rabbit antiserum to a fraction (B1), which contained in relatively purified form most of the toxin in pilot plant ricin, were subsequently injected with fraction B1 and with fraction B3, a gummy fraction presumably containing much castor bean allergen but virtually free of ricin itself. Injection of fraction B1 uniformly produced fatal anaphylactic shock, whereas injection of fraction B3 produced much less severe reactions. The animals which received fraction B3 were fatally or severely shocked by subsequent injections of B1.^{3,18k}

2. All guinea pigs that had been immunized by injections of ten times recrystallized ricin, of pilot plant ricin, or of alum-precipitated ricin toxoid showed anaphylactic responses when injected intravenously with crystalline ricin or when exposed to airborne pilot plant or crystalline ricin at relatively low concentrations.^{3,17d,18t}

3. Guinea pigs injected with ricin develop considerable immunity to the toxic effects of ricin (Section 12.4.3). In general, immunity in guinea pigs goes hand in hand with hypersensitivity.

Since the completion of this work, Canadian investigators⁵³ have reported failure of attempts to elicit anaphylactic responses in guinea pigs sensitized with crystalline ricin and exposed to airborne pilot plant or crystalline ricin. Animals immunized with pilot plant ricin showed weak responses. On the other hand, animals given a single injection of castor bean allergen^{66,67} reacted vigorously to crystalline ricin as well as to pilot plant ricin in low concentrations. These data, considered in conjunction with supplementary results obtained by the use of the Schultz-Dale technique, led to the conclusions that

^g The sensitivity claimed for the hemagglutination reaction in reference 27 appears to be in error.

the sensitization was to allergen rather than to toxin, and that allergen is present in crystalline ricin. Additional evidence that a small amount of allergen is present even in many times recrystallized ricin has since been presented.¹⁴

Further work is required to clarify the apparent discrepancies. In any event, it is evident that guinea pigs can be prepared in such a way as to render them highly susceptible to anaphylactic shock upon exposure to very low concentrations of all known ricin preparations.

The impracticability of employing the reactions of animals other than dogs as routine methods of detection in warfare has often been emphasized. However, general considerations as well as the results obtained during field trials with ricin^{3,22} would indicate that sensitized guinea pigs could be of great value in the hands of special officers assigned the duty of checking upon the possible use of protein toxins by an enemy.

12.5

ASSAY^b

It was early recognized that the toxicity of castor beans was associated with the water-soluble, heat-coagulable protein of the beans.^{64,65} The presumption was that the toxicity was the unique property of a single protein component, and this hypothetical component was designated ricin. In 1943, a crystalline protein was isolated from extracts of castor beans.^{2,15} Its toxicity was reproducible and was about twice as great as that of the most active amorphous product available.^{9,15} This result greatly strengthened the presumption that there is present in castor beans a single toxic protein component. However, although the crystalline product has met some, it has not met all, of the criteria of molecular homogeneity which are required of a single protein.^{2,3,15} The possibility cannot yet be rigorously excluded that the toxin is a complex whose components may, some day, be separated from one another and may then be found to be active only in association with one another. As long as this possibility remains, the only assay of the toxin of castor bean preparations to which no objection can be raised is an estimation of the toxicity under approved experimental conditions. Theoretically, the measurement of any physical, chemical, or biological property which has been shown to be quantitatively related to the toxicity should serve as an assay. Unfortunately, the demonstration of the existence of

such a relation cannot be complete until the pure toxin has been isolated and fully characterized. A variety of properties of extracts of castor beans have been proposed as bases for assay and these will be reviewed briefly. The problem of bioassay will, however, be considered first and in fuller detail.

12.5.1

Bioassay

All vertebrates that have been tested are susceptible to ricin (see Section 12.3). Few observations have been made on invertebrates. It has been reported⁵ that the motility of certain species of protozoa is arrested by the addition of ricin to the medium, but it has not been established that the potencies of a series of preparations of ricin are proportional to their toxicities. Difficulties in controlling the action on the protozoa led to abandonment of the attempt to use these organisms as a means of assay.

Ricin acts slowly on vertebrates. With minimum lethal doses, animals seldom die in less than 5 or 6 days, and may survive for weeks. Assays based upon the estimation of median lethal doses are, therefore, protracted and require an arbitrary choice of observation period. They also require large numbers of animals for statistical validity because of incidental variables such as casual infections. Where many assays must be carried out, there are obvious advantages in the adoption of a method which gives quick results and is economical of animals.

The value of establishing a relation between the dose of ricin and the survival time in a given species as a basis of a method of assay was urged in 1918.²⁴ In this country the problem has been investigated in three different laboratories.^{5,9,15,61} Work was also done in Canada⁴² and in England.^{33,37} The mouse has been the favored animal, being preferred to the rat.⁴² Several homozygous strains have been used,^{5,9} the most popular one in the United States being the CF1 strain of white mice developed by Carworth Farms, New City, Rockland County, New York.^{5,9,15}

A method of assay based upon 24-hour mortalities has been adopted. Groups of five or preferably ten mice weighing 20–25 g are injected with a series of graded doses by the intraperitoneal route. The individual survival times of the animals are recorded and the mean survival time for each dose is derived. By interpolation in an accepted dose-survival time relationship, a value for the dose corresponding to a mean survival time of 24 hours is, then, obtained for each experimental dose. This 24-hour lethal dose has

^b By R. Keith Cannan.

been designated the toxicity unit [TU]. Because of the skewness of the distribution of survival times, one investigator⁵ prefers to convert each individual death time in a group to a TU and to average these to give the TU for the group.

It has been customary to express the TU in micrograms of the preparation per 20-g mouse though it is sometimes more useful to express it in terms of the total nitrogen or the coagulable nitrogen present in the preparation. The desirability of making a simultaneous assay of a standard product has been emphasized by all laboratories concerned with the evaluation of ricin preparations.^{5,9,15} When this is done, one may then readily express the toxicity of an unknown preparation in terms of the per cent of the standard which it contains. Crystalline ricin is the logical reference standard.

No difference in susceptibility to ricin of the two sexes of the CF1 strain of mice has been detected.⁹ In the cases of two other strains, small differences have been recorded.^{5,42} Lean mice seem to be more resistant than fat mice of the same weight.^{5,42} This is probably because a greater proportion of the body weight of the lean animals is active tissue. Studies of the effects of diet⁵ also have led to the conclusion that the toxicity is a function of the ratio of active tissue to body weight. Mice to be used for assay should be free from parasites. The susceptibility of young mice is found to be greater when the environmental temperature is elevated and is reduced at low temperatures.^{5,42} This is presumably due to corresponding changes in body temperature. Frogs are also sensitive to ricin only at elevated temperatures.⁶⁵ In the conduct of assays with mice, the temperature at which the mice are kept should be controlled. The preferred temperature has been close to 25 C.

In one laboratory,⁵ automatic devices for the control of temperature, the injection of the animals, and the recording of individual death times have been employed with the object of rendering the conditions of assay as uniform as possible.

If the preparation of ricin is very active, it must be diluted to a concentration of 10–50 mg per liter before injection. The danger has been emphasized⁵ of losses by adsorption on the walls of the vessels in which the solution has been prepared. Adsorption is said to be significant even when paraffin-coated vessels are used. To reduce this error, a solution of 0.3 per cent egg albumin has been used as the diluent on the principle that the excess of inert protein will inhibit the adsorption of the toxin.⁵ Shorter sur-

vival times are observed when this procedure is adopted,^{5,9,15} but there has been debate as to whether this was due to the suppression of adsorption or was the result of a synergistic action of the egg albumin. The modified technique has not found general acceptance.^{9,15} When a simultaneous assay of crystalline ricin is made and the result is expressed as the per cent of crystalline ricin in the product, it is probably immaterial whether water or 0.3 per cent egg albumin is used as the solvent. On the other hand, toxicity unit values derived from assays of egg albumin solutions of ricin are consistently smaller than those of aqueous solutions and are not directly comparable with them.

THE DOSE-SURVIVAL TIME CURVE FOR MICE

For a given route of injection, this curve approximates a rectangular hyperbola of the type represented by the relation:^{5,9,15}

$$\frac{(D - D_m)}{(t - t_m)} = k,$$

where D is the observed dose, t is the observed survival time, and k is a constant characteristic of the preparation of ricin. D_m and t_m are constants having the qualities of an extrapolated minimum lethal dose and an extrapolated minimum survival time respectively. Over a wide range of lethal doses a single pair of values of D_m and t_m fits the experimental observations only very roughly. Over restricted ranges of survival times, however, values of the constants can be so chosen as to fit the data quite satisfactorily.⁹ For short survival times, D_m is small relative to D and may be ignored. Then, since the toxicity unit is the value of D when $t = 24$ hours, the above equation can be rewritten in the form:

$$\text{TU} = D \frac{(24 - t_m)}{(t - t_m)}.$$

This assumes that t_m is independent of the nature of the material which is being assayed. If this assumption is not correct (and it has been implicitly questioned)⁵ the assay of an unknown product by interpolation in a standard curve would be subject to error. However, extensive observations^{9,15} have indicated that the use of $t_m = 13$ hours satisfies the intraperitoneal data for both crystalline ricin and standard ricin over a range of survival times of about 18 to 30 hours. These laboratories have, accordingly, adopted the following equation for general use:

$$\frac{TU}{D} = \frac{11}{(t - 13)}.$$

In order that the uncertainty of interpolation should be minimized, it is recommended that values of TU should be computed only from mean survival times falling within the limits of 21 and 28 hours.

A summary of the data of one laboratory⁹ on crystalline ricin and standard ricin is given in Table 7. In Table 8 will be found a comparison of the results

TABLE 7. Summary of a series of assays of crystalline ricin and of standard ricin carried out at intervals over a period of 13 months.⁹ The CF1 strain of mice was used. Solutions were prepared and diluted with water.

	Crystalline ricin		Standard ricin	
	Male	Female	Male	Female
Total number of assays	23	22	13	12
Mean toxicity unit μg of material per 20-g mouse	2.00	2.04	6.96	7.04
Standard deviation	0.21	0.175	0.79	0.72

TABLE 8. Dose-survival time relations for different routes of injection. The CF1 strain of mice was used. Solutions were prepared in water and injected in a volume equal to 1 per cent of the body weight.

Standard equation: $D(t - t_m) = k$.

	Intravenous	Intraperitoneal	Subcutaneous
k (μg-hours)	8.6	23	50
t_m (hours)	11	13	16
TU μg/20 g	0.66	2.1	6.3
LD ₅₀ μg/kg	2.2	10.4	22.1

of intravenous, intraperitoneal, and subcutaneous injections.⁹ The method of intravenous injection is technically too difficult for routine assays. All investigators have agreed that intraperitoneal injections yield more precise and reproducible results than do those by the subcutaneous route. Subcutaneous toxicities have been found to vary to a remarkable degree with the concentration of the solution which is injected.⁹

12.5.2 Alternative Methods of Assay

A variety of properties of ricin preparations have been proposed as bases for assay. These include the antigenic properties, the hemagglutinating potency, and various enzymic activities which have been found in crude preparations of ricin. The weight of evidence is that none of these are specific for the active toxin. Some of them are valuable for the comparison of limited types of preparation, but must be supplemented by bioassays in critical situations.

THE QUANTITATIVE PRECIPITIN METHOD³

Antiserums prepared by the injection of crystalline ricin have been found to precipitate from extracts of castor beans, and amorphous preparations generally, not only the toxin, but a nontoxic protein.^{3,15} Since this material is antigenically indistinguishable from the toxin, it may be called a natural toxoid. In the preparations that have been tested, the ratio of toxin to toxoid has varied from 1/1 to 2/1. Only by the process of crystallization has a separation of the two antigenic components been accomplished. If this limitation of the method is borne in mind, the precipitin technique (see Section 12.4) is a valuable method of assay. It requires only a few micrograms of purified material and gives a positive result within a short time.

HEMAGGLUTINATION

There is a wealth of evidence that the hemagglutinating activities of ricin preparations do not parallel their toxicities.^{3,5,15,24,34} Crystalline ricin has, for example, only about 20 per cent of the agglutinating power of amorphous preparations which are considerably less toxic.^{3,15} The method can, therefore, have only limited use.

A method of assay based on hemagglutination (see Section 12.4) has been proposed for use in the field.^{22,23,26} Agglutination tests are rapid and require only small samples of material. However, the customary method of evaluating the agglutinating potency of a sample is only coarsely quantitative and depends on subjective discrimination by the observer. An attempt to increase the objectiveness and precision of the method has been described.¹⁵

ENZYMIC ACTIVITY

The observation that crystalline ricin does not exhibit the esterase, phosphatase, and lipase activities of crude preparations eliminates these properties as means of assay.⁵ It has recently been reported^{43,50} that ricin preparations hydrolyze adenosine triphosphate. If it should be established that this activity is proportional to the toxicity in a representative series of preparations, a valuable alternative to bioassay may become available.

CHEMICAL METHODS

No chemical property of the toxin is known which distinguishes it from other heat-coagulable water-soluble proteins of the bean. However, water extracts from castor bean contain little coagulable pro-

tein other than the toxin and the toxoid.^{9,15} In such extracts, an estimation of the heat-denaturable protein gives a result only slightly greater than the estimation of the protein precipitated by antiserum.^{9,15} In extracts prepared with salt solutions, on the other hand, a large additional amount of coagulable protein is present.^{15,34} Fortunately this is denatured at or below pH 4. If such extracts are acidified and filtered, the soluble coagulable protein which remains corresponds closely to the sum of the toxin and toxoid. The heat-coagulable protein has usually been estimated as the difference between the soluble protein before and after boiling for 15 minutes to 1 hour at 100 C. Any acceptable method of protein determination may be used which is adapted to the amount of protein present.¹⁵

12.5.3 Field Detection and Assay

For the rapid detection of airborne ricin, the sensitized guinea pig is undoubtedly the most sensitive and specific^{3,22} (see Section 12.4). The maintenance and care of sensitized animals in the field, however, present many difficulties. Moreover, there is some question whether the anaphylactic reaction is elicited by the toxin or by a so-called allergen which may be separated from it.^{5,53}

Any other method of detection or assay requires the collection of samples adequate in amount for the test which is to be performed. Certain color tests have been proposed and are both sensitive and rapid.⁵ In so far as they are simply tests for protein or for the carbohydrate commonly associated with protein, they are entirely nonspecific and are of value only in indicating the possible presence of ricin. More specific, but less suited to field work, are the hemagglutination and precipitin tests. Some limitations of the former have been mentioned. The precipitin reaction is decidedly more specific and more accurate. It has, however, been pointed out that the heavy metals present in some smokes will give nonspecific precipitates with serum proteins. Neither test gives an immediate response. An assay of toxicity is, of course, the most dilatory of all.

It may be appropriate, in conclusion, to remind the reader that the hazard of exposure to a non-volatile airborne toxin cannot be evaluated simply from the time of exposure and the concentration of toxic material in the cloud. The inhalation toxicity is determined in large measure by the particle size distribution in the cloud. The relation of particle size to toxicity is discussed in Section 12.3 and in Chap-

ter 15, where methods of evaluating the particle size distribution in a cloud are reviewed. In contemporary field trials²² with standard ricin, it was found profitable to assay the cloud not only for toxicity and for particle size but also for total protein and for heat-coagulable protein. The two latter estimations provided useful information on the extent to which the method of dispersal resulted in detoxification and denaturation of the material with which the munitions had been charged. The results of field trials are reviewed in Section 12.6.

12.6 EVALUATION AS A WAR GASⁱ

The performance of field trials on munitions charged ricin and the interpretation of the results of these trials in terms of evaluation of the agent as a war gas rest in large part on the laboratory researches in the United States, Canada, and Great Britain on particulate sampling and bioassay (Chapter 15).

The most significant criterion for effectiveness of ricin in the field was bioassay by animals exposed to the particulate cloud. Physical measurements were essential to an understanding of the reasons for poor or good results in the several trials and as a guide for design of subsequent trials. Low toxicity in the field could be associated with many variables, including large particle size arising from compaction and aggregation, thermal inactivation of the sensitive protein agent, inefficient munition functioning, and meteorological conditions. The field trials also rested on the prior development of pilot plant methods for the preparation of finely divided ricin (Section 12.2).

12.6.1 Relative Efficiency of Dispersion by Different Munitions

The principal types of munitions and chargings which have been studied for the dispersion of ricin are the following:

1. High explosive-chemical bombs charged with a suspension of ricin in carbon tetrachloride. Bombs of this type, with steel casings and axial bursters, were employed in the British experiments carried out in 1941^{33,36} and in the recent Canadian trials.⁴⁶ Munitions of this type retain to a significant degree the effectiveness of ordinary HE fragmentation bombs.

2. Light-case metal bombs charged with dry ricin. The Canadian 4 lb L.C. bomb was a metal can holding about 550 g of ricin and fitted with a small burster

ⁱ By Stanford Moore.

(e.g., 20 g of nitroguanidine and 70 g of sodium bicarbonate).^{22,43,44}

3. Base ejection bombs charged with dry ricin. The U.S. M-74 10-lb tail ejection incendiary bomb was modified for use with about 385 g of dry ricin.^{22,25}

4. Gas ejection bombs charged with dry ricin. NDRC Division 10 carried out developmental work on a two-compartment bomb holding liquid carbon dioxide or compressed air in one compartment, which on functioning ejected the particulate charging from the second compartment.²²

5. Plastic and glass bombs charged with a suspension of ricin in carbon tetrachloride. Experimental munitions of this type developed by NDRC Division 10 were similar to (1) above but with plastic or glass casings instead of steel.⁴⁵

The lines of investigation on dispersion of ricin from the various types of bombs at the several field experimental stations have led to the same general conclusions. The results indicate that high explosive-chemical bombs charged with a 35 per cent suspension of ricin in carbon tetrachloride are superior to the dry powder munitions in their ability to put up a cloud in which the volume mass median diameter is sufficiently small to pass the nasal barrier.^{22,33,36,37,45,46} This conclusion confirms the earlier analysis of the problem made by British investigators in 1941^{33,36} on the basis of a less complete series of experiments.

In the field trials plastic bombs have given results comparable with those obtained with steel bombs but the British 4-lb HE/Chem Type F Mk I steel bomb, as used in the later Suffield trials, possessed the advantages of availability in standard design and durability in transport.

The lower dispersion efficiency of the munitions charged dry powdered ricin was largely the result of the formation of aggregates in the particulate clouds.²² Comparisons were based on parallel tests employing a given sample of powdered ricin set up both in the dry and suspension forms.⁴⁵ In trials with dry samples, aggregation of the initial particles of the ricin charging to yield a cloud of larger mass median diameter was increased by increase in the moisture content of the charging or in the relative humidity of the atmosphere.²²

In the 1941 British trials^{33,36} it was concluded that bombs filled with ricin suspended in carbon tetrachloride were at least three times as effective as similar bombs filled with a solution of ricin in water. In more recent tests with plastic bombs the solutions in

water were also found to be less stable to detonation than suspensions in carbon tetrachloride.^{17c,d,f} The munitions were functioned in a stainless-steel explosion chamber at the NDRC University of Chicago Toxicity Laboratory and a material balance determined. No measurable denaturation was observed in the case of the suspensions in carbon tetrachloride, whereas a 40 per cent loss in toxicity occurred with the aqueous ricin solutions. Chamber trials on the plastic munitions were also carried out at the Division 10 NDRC Munitions Development Laboratory.^j

12.6.2 Comparison with Bombs Charged Phosgene

On the basis of the early trials with suspensions of ricin in carbon tetrachloride the British investigators concluded in 1941^{33,36} that bombs filled with ricin were about as effective as phosgene bombs of the same size. With improvements made in the pilot plant manufacture of dispersible ricin since that date, and progress in the testing of munitions, the relative effectiveness of ricin has been increased to a position well above that of phosgene. The comparative data have been analyzed by the Suffield Experimental Station.⁴⁶ From calculations of the dosage contours from the field trial data the munition expenditures required for 80 per cent coverage of a target area with a ricin dosage of at least 100 mg/min/m³ have been calculated. The $L(Ct)_{50}$ of ricin for man is not known. The results of the field experiments indicate that for goats in the field the $L(Ct)_{50}$ of the present pilot plant samples of ricin dispersed by the 4-lb HE/Chem Type F bomb is about 100 mg/min/m³. For the present calculations it is assumed that this value holds for man. Employing the methods of calculation applied to the test data on phosgene³² it is estimated that for 500-lb clusters of Type F bombs an expenditure of 1.2 clusters (43 lb of ricin) per 100x100 yard square would cover about 80 per cent of the target area with an $L(Ct)_{50}$ dosage on open terrain (neutral temperature gradient; wind speed less than 12 mph). For 500-lb bombs charged phosgene under the same conditions the estimated expenditure is 8 bombs (1,600 lb of phosgene) per 100x100 yard square for coverage by a dosage of 3,200 mg/min/m³ within 30 seconds or 4 bombs for coverage within 2 minutes. The comparison is based on tests with a batch of spray-dried

^j These are reviewed in the Summary Technical Report of Division 10.

air-ground ricin with a volume median diameter of 3.3μ which yielded clouds of volume mass median diameter of about 15μ .

From this it is concluded that ricin appears to be at least seven times as effective as phosgene on the basis of aircraft stowage when the comparison is based on a 30-second dosage of phosgene. If the $L(Ct)_{50}$ for phosgene is considered to be high by even a factor of two there would still be a margin in favor of ricin. Since ricin in carbon tetrachloride gives no detectable odor, the comparison on the basis of a 30-second dosage is suggested as the fairest comparison. On the basis of weight of active agent employed, rather than the weight of munition, ricin has a superiority over phosgene of 40 to 1 from these data.⁴⁶

12.6.3 Ricin as a War Gas

Ricin is an odorless powder capable of being dispersed as a particulate or dust cloud. The absence of odor and the complexity of the consequent detection problem in the field would render ricin more insidious than any standard U. S. or British chemical warfare agent. Comparison with the German Trilons (Chapter 9) would present a closer differentiation problem. The physiological effects of ricin are delayed. Lung injury, similar in character to that produced by phosgene, can lead to deaths at from one to several days after exposure. Ricin can be dispersed in munitions not readily distinguishable from standard HE bombs.

For detection in the field attention has been given to hemagglutination tests and to the use of ricin-sensitized guinea pigs (Section 12.4). These methods are intrinsically more difficult in practice than the

simple means for detecting such agents as mustard gas or phosgene by odor or chemical tests. The U. S. and British gas masks, when well adjusted, give complete protection against any dosage of ricin likely to be produced in the field.³⁷ The immunization of troops against ricin and serum therapy present difficulties, as outlined in Section 12.4.

From the few tests on the persistence of ricin in the field it has been concluded that the major part of the agent is rapidly dissipated in the particulate cloud. Only in the area immediately around the point of burst was ground contamination sufficient to be measurable. In tests in which sensitized guinea pigs were allowed to run through the brush in this area a possible hazard was detectable for about 3 days in dry weather.²²

As a result of the progress made during World War II on the preparation and dispersion of ricin it must be considered that in all-out chemical warfare it is possible that ricin could be employed in a practical role in chemical munitions. Supply and manufacture would place a ceiling on the scale of use but would not prevent the accumulation of significant quantities of this agent. It has been estimated that the cost of production of dispersible ricin on a large scale would be approximately \$13 per pound (Section 12.2).

In the course of the research during World War II the work on ricin has served to advance the knowledge on the general problem of particulate dispersion. In some respects ricin has served as a model substance for work on the dispersion of agents of similar chemical and physical properties in the related research in the field of bacteriological warfare.

Chapter 13

AROMATIC CARBAMATES

By Arthur C. Cope

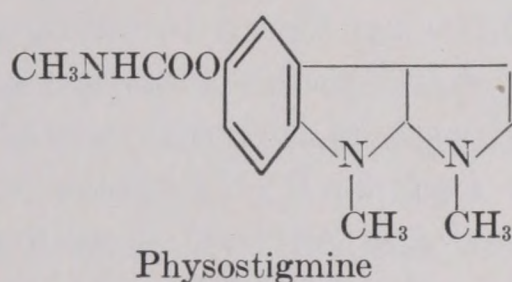
13.1

INTRODUCTION

BEGINNING IN 1943 under the auspices of the National Defense Research Committee [NDRC], search for a superior nonvolatile toxic agent was undertaken by several cooperating laboratories. Criteria for the agent sought were extreme toxicity on subcutaneous injection, rapid lethal action, ready availability through practical synthesis or otherwise, and sufficient stability for military use and storage.

A survey of the open literature¹⁷ and information currently available concerning the toxicity of chemical warfare agents guided the search. Among the more toxic classes of substances known, botulinus toxin, other bacterial toxins, and potent plant toxalbumins (particularly ricin) were considered unsuitable because of slowness of their toxic action, immunological characteristics, and in some cases inadequate sources of material for possible use on a considerable scale.

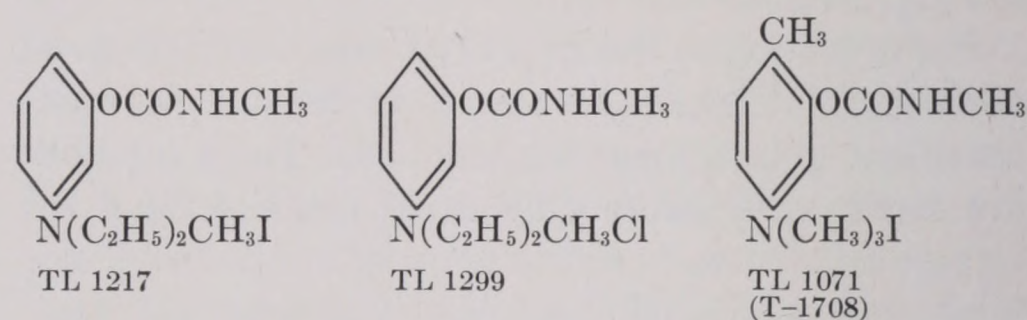
The alkaloids physostigmine and aconitine were high on the list of toxic substances.



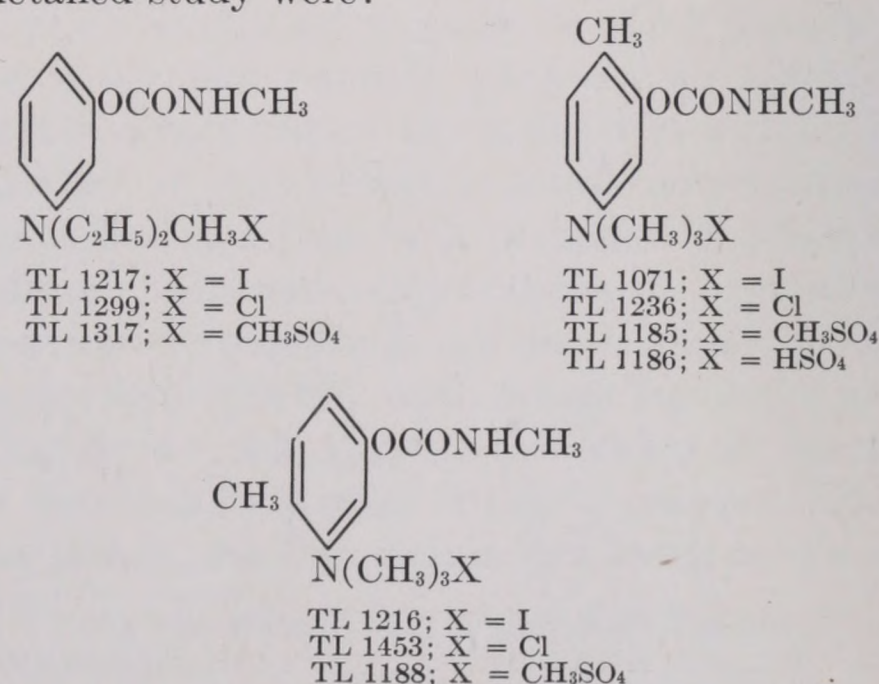
Aconitine is the more toxic of the two, but its complete structure is unknown, and search for a toxic agent among simpler related compounds is unpromising because minor structural modification of the toxic aconite alkaloids often destroys their toxicity. On the contrary, many synthetic N-alkylcarbamates related to physostigmine are highly toxic, and search for a superior agent in this class appeared more promising. For this reason, and after failure to obtain highly toxic compounds in several other classes, the investigation soon turned to a thorough exploration of the carbamates. Similar studies were conducted at an earlier date in England by R. D. Harworth and his associates, and in Canada by Leo Marion and others. The following investigations of carbamates reported in the open literature preceded all the classified work.

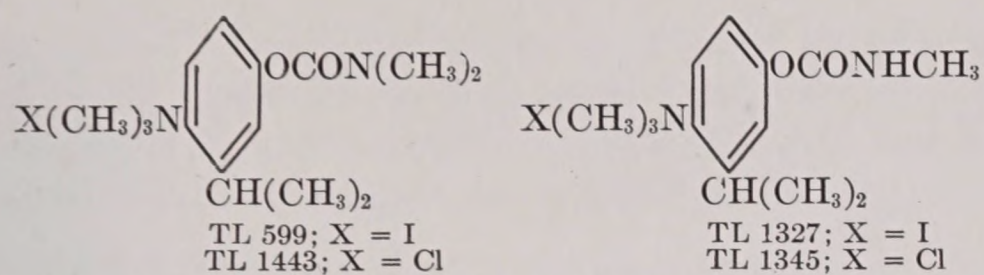
After establishment of the structure of physostigmine by Stedman and Barger,⁴² a number of synthetic analogs were prepared by Stedman. Several of his papers⁴³⁻⁴⁷ describe the synthesis and miotic properties of such analogs, but contain no toxicological information. White and Stedman⁴⁸ report a detailed pharmacological study of miotine, the synthetic miotic of choice from the group, including toxicity data for this substance and three related carbamates. Aeschlimann and Reinert⁴⁹ report toxicity and other pharmacological data for physostigmine and 44 related synthetic carbamates, many of which had been prepared earlier by Stedman. Stevens and Beutel⁵³ also have investigated physostigmine substitutes, and report chemical and toxicity data for 27 related carbamates. The toxicity data for such compounds are recorded in the open literature.^{48,49,53}

As a result of the classified British and Canadian work, TL 1071 (British code T-1708) was the leading candidate in the carbamate group. The compounds TL 1217 and TL 1299 proved to be the agents of choice on the basis of the NDRC work.



Additional compounds that received more or less detailed study were:



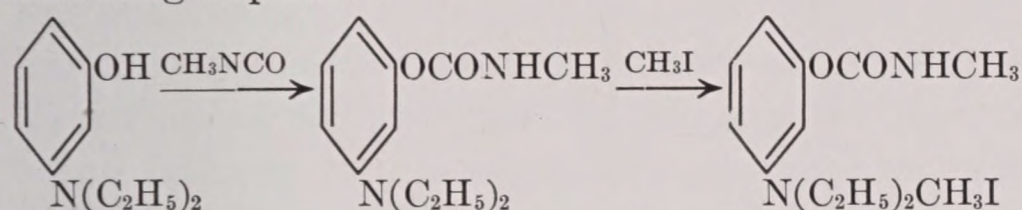


In the following pages work on the carbamates which might be of some practical importance as toxic agents is summarized. Investigations which led to selection of leading candidates are mentioned briefly.

13.2 SYNTHESIS

13.2.1 *m*-Diethylaminophenyl-N-methylcarbamate methiodide (TL 1217)

The most practical preparation of TL 1217 is the following sequence:



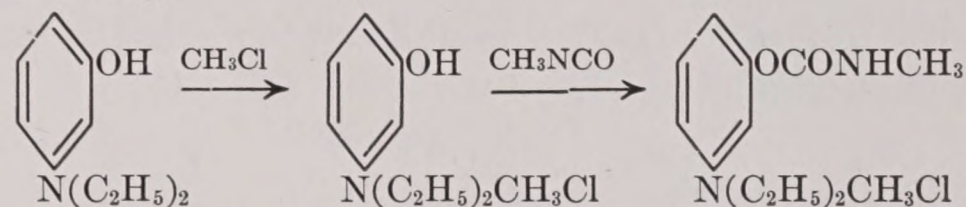
This process was operated successfully on a pilot plant scale.¹⁵ Approximately 360 lb of methyl isocyanate were prepared by reaction of methylamine and phosgene in the vapor phase to give methyl carbamyl chloride, which was converted to methyl isocyanate by treatment with pyridine in toluene. The average yield was 81 per cent. *m*-Diethylaminophenol (a commercial dye intermediate) dissolved in dry benzene was refluxed with an excess of methyl isocyanate for several hours. *m*-Diethylaminophenyl-N-methylcarbamate was isolated in yields of over 80 per cent by evaporating the solvent under reduced pressure, filtering, washing, and drying. TL 1217 was prepared by reaction of *m*-diethylaminophenyl-N-methylcarbamate with methyl iodide in acetone under reflux. After addition of ethyl acetate, the product was recovered by filtration in yields of 79 to 86 per cent. The product so obtained was of high purity, as verified by elementary analyses, use of a special analytical procedure involving hydrolysis and determination of carbon dioxide and methylamine,¹³ and toxicity tests.

Essentially this same procedure had been used earlier for the preparation of TL 1217 on a laboratory scale.^{1,2,5} This compound is among the group described by Aeschlimann and Reinert⁴⁹ and by R. D. Haworth.²³ Prior to development of a practical synthesis of methyl isocyanate, *m*-diethylaminophenyl-N-methylcarbamate was prepared on a large laboratory scale in yields of 74–86 per cent by reaction of *m*-diethylaminophenol with phosgene in the presence

of diethylaniline, followed by reaction with methylamine.^{2,3,14} This procedure was developed from a similar method used by Marion.^{29,32}

13.2.2 *m*-Diethylaminophenyl-N-methylcarbamate methochloride (TL 1299)

The most practical preparation of TL 1299 is the following:



This process was operated successfully on a pilot plant scale.¹⁵ Redistilled *m*-diethylaminophenol was treated with an excess of methyl chloride in an autoclave at 100 C. After cooling and evaporation of the excess methyl chloride, the product was purified and isolated in 77 per cent yield by grinding, washing, and drying. Yields in this step on a laboratory scale were 95 per cent.¹³ *m*-Diethylaminophenol methochloride was converted to TL 1299 by reaction with methyl isocyanate in dimethylformamide as a solvent and a mixture of triethylamine and glacial acetic acid as a catalyst. Yields on a pilot plant scale were 91 per cent.¹⁵ This process was developed to a high state of perfection in an intensive laboratory investigation,¹³ in which yields of 94–97 per cent and 90–92 per cent in the two steps were obtained, or 86–90 per cent overall. A useful laboratory synthesis of methyl isocyanate from methylamine and phosgene also was developed in this work,¹³ and was used until it was superseded by the pilot plant process¹⁵ for this essential intermediate.

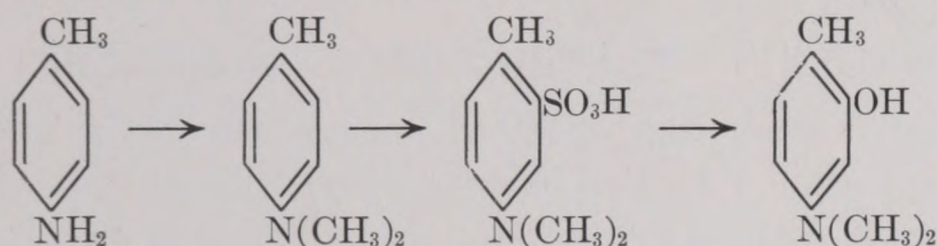
Prior to development of the above process, TL 1299 was prepared by a different procedure. *m*-Diethylaminophenyl-N-methylcarbamate was prepared first from *m*-diethylaminophenol by the phosgene-methylamine procedure, with diethylaniline as the acid acceptor (yield 78 per cent).^{2,3,14} This product was converted to the methosulfate salt (TL 1317) by reaction with methyl sulfate (yield 75–79 per cent), and TL 1317 was converted to TL 1299 through reaction with anhydrous calcium chloride in methanol containing hydrogen chloride (yield 74 per cent).^{2,3} Earlier TL 1299 was prepared in high yields from TL 1217 and silver chloride.^{2,3,5,14}

13.2.3 (2-Methyl-5-dimethylaminophenyl)-N-methylcarbamate methiodide (TL 1071)

TL 1071 (British code T-1708) was commonly called the "Haworth compound" during the NDRC

investigations, since it was the leading candidate from the British work. Details of Haworth's method of preparation could not be obtained, but Canadian reports on the synthesis^{26,35} were available and served as a basis for further developments.

The intermediate 2-methyl-5-dimethylaminophenol was obtained from the National Aniline Division of the Allied Chemical and Dye Corporation, where it was prepared from *p*-toluidine by methylation, sulfonation, and alkaline fusion:

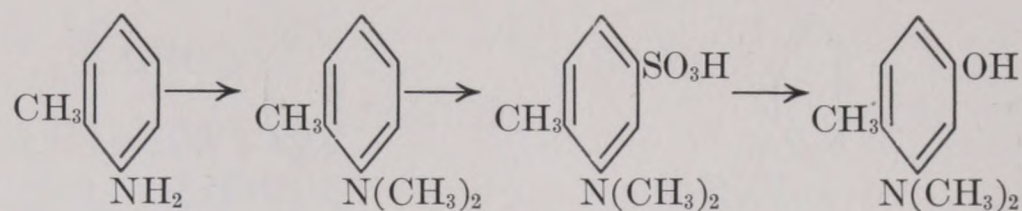


2-Methyl-5-dimethylaminophenol was converted into the N-methylcarbamate by treatment with phosgene in the presence of diethylaniline, followed by methylamine (yield 75–80 per cent).¹⁴ With methyl isocyanate available,¹⁵ this intermediate could be used in preparation of the N-methylcarbamate, which has been prepared in that manner in 85 per cent yield on a small scale.⁵ TL 1071 was prepared from the N-methylcarbamate and methyl iodide in acetone in 95 per cent yield.¹⁴ In a Canadian pilot plant operation, 39 lb of TL 1071 were prepared from 2-methyl-5-dimethylaminophenol by this process, with an overall yield of 39 per cent.³³

Other quaternary salts differing from TL 1071 only in the anion were prepared. Among these were the methosulfate, TL 1185,¹⁴ which was hydrolyzed slowly with aqueous hydrochloric acid or water to the acid sulfate, TL 1186.¹⁴ The latter on treatment with calcium chloride yielded the methochloride, TL 1236. The preferred procedure for preparing this compound was to heat the crude methosulfate with an alcoholic solution of calcium chloride for 20 hours. Overall yields from the N-methylcarbamate were 77–87 per cent.¹⁴ Treatment of the N-methylcarbamate with methyl chloride also yielded TL 1236.⁵

13.2.4 (4-Methyl-3-dimethylaminophenyl)-N-methylcarbamate methiodide (TL 1216)

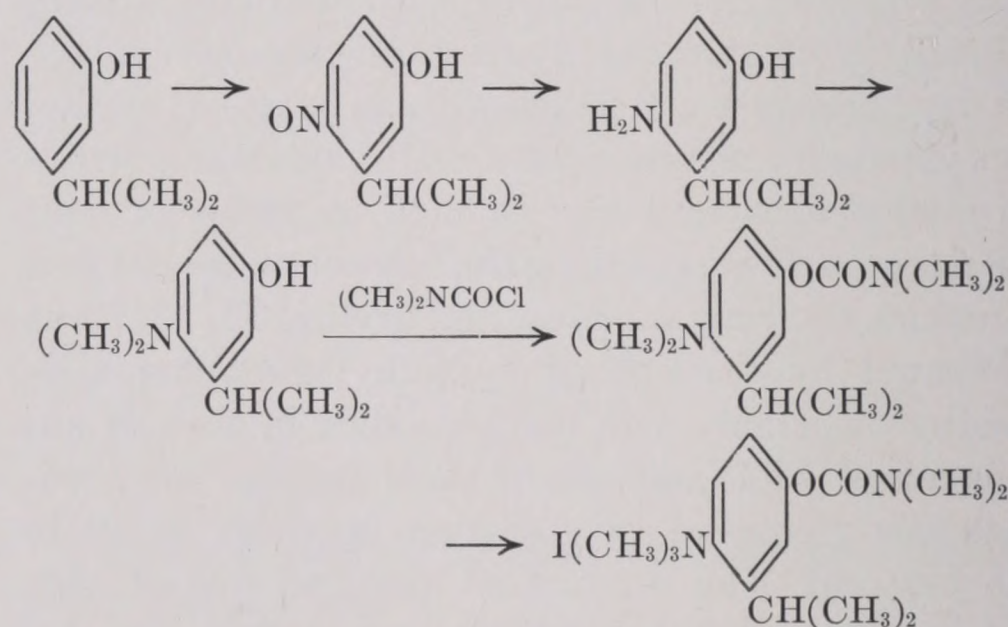
TL 1216 was prepared during the Canadian work,²⁷ and became known during the NDRC investigations as the Haworth isomer. It was synthesized in Division 9, NDRC, from 4-methyl-3-dimethylaminophenol, which was prepared by the National Aniline Division of the Allied Chemical and Dye Corporation from *o*-toluidine:



Both the phosgene-methylamine procedure^{14,27} and methyl isocyanate⁸ were used in preparing the N-methylcarbamate. The methiodide, TL 1216, was prepared from the N-methylcarbamate.^{8,14} Other salts prepared were the methochloride⁸ (TL 1453) and the methosulfate (TL 1188). Synthetic methods employed paralleled those described for TL 1071.

13.2.5 (3-Isopropyl-4-dimethylaminophenyl)-N,N-dimethylcarbamate methiodide (TL 599)

TL 599 is the most toxic of the carbamates described by Stevens and Beutel.⁵³ Its preparation on any scale is hindered by lack of a practical source or synthesis for *m*-isopropylphenol, the essential starting material. An investigation of eight routes to this compound was made,¹⁹ of which the most satisfactory started with benzoic acid and continued through methyl *m*-hydroxybenzoate and *m*-hydroxyphenyldimethylcarbinol, by way of the Grignard reagent. The remaining steps in the synthesis of TL 599 were the following:¹⁹



Compounds in the corresponding N-monomethylcarbamate series also were prepared (TL 1327, TL 1345).^{9,19}

13.2.6 Synthesis of Other Aromatic Carbamates for Toxicity Tests

In addition to the carbamates described in the preceding sections which were the subject of relatively intensive laboratory or pilot plant investigations, many similar compounds were prepared on a small scale for toxicity tests, in the search for the most toxic and readily synthesized agent in the

group. The following references contain the results of such investigations, and an indication of the classes of compounds studied where that information can be stated concisely; otherwise they are classified as miscellaneous.

Classes of Carbamates Described	Ref. No.
Miscellaneous; sulfur analogs	1
Derivatives of polyhydric phenols	4
Derivatives of 3-diethylaminophenol, 3-dimethylaminophenol, 2-methyl-5-dimethylaminophenol and 2-methyl-5-diethylaminophenol	5
Derivatives of 4-dimethylaminothymol and 4-dimethylaminocarvacrol	6
Homologs and analogs of Doryl (aliphatic carbamates)	7
Derivatives of <i>p</i> -aminophenol, 4-methyl-3-aminophenol, 3-methyl-4-aminophenol, 2-methyl-5-aminophenol	8
Derivatives of 5,6,7,8-tetrahydronaphthol-1; 3-isopropyl-4-aminophenol; miscellaneous	9
Derivatives of 3,5-dimethyl-4-aminophenol	10
Derivatives of 3-alkyl-4-aminophenols	11
TL 1299, the corresponding N,N-dimethylcarbamate methiodide (TL 1238) and methochloride (TL 1422)	13
Derivatives of 2-methyl-5-dimethylaminophenol, 4-methyl-3-dimethylaminophenol, 2-methyl-5-diethylaminophenol, <i>m</i> -diethylaminophenol	14
Derivatives of 3-isopropyl-4-aminophenol, 2,6-diisopropyl-4-aminophenol, 2-isopropyl-5-aminophenol, 4-isopropyl-3-aminophenol, 4-isopropyl-2-aminophenol; arsenic analogs	19
Miscellaneous; toxicity data only on compounds prepared by R. D. Haworth	23
Miscellaneous	24
Derivatives of <i>m</i> -dimethylaminophenol	25
Derivatives of 2-methyl-5-dimethylaminophenol	26
Derivatives of 4-methyl-3-dimethylaminophenol	27
Derivatives of <i>m</i> -diethylaminophenol	29
Derivatives of 2,4-dimethyl-5-dimethylaminophenol	31
Miscellaneous	34
Miscellaneous	43
Miscellaneous	44
Miscellaneous	45
Miscellaneous	46
Miscellaneous	47
Miscellaneous	48
Miscellaneous	49
Miscellaneous	50
Miscellaneous	51
Miscellaneous	52
Miscellaneous	53

13.3

STABILITY

The toxic aromatic carbamates of possible practical importance are reasonably stable at 65 C, showing little decomposition after 2 months storage.¹⁶ The two labile groups in such compounds are the carbamate and quaternary salt linkages. The carbamate group is subject to thermal decomposition to methyl isocyanate and the corresponding phenol, and to hydrolysis to the phenol, methylamine, and carbon dioxide, or related products. The quaternary

salt groups are subject to decomposition at elevated temperatures to an alkyl halide and the corresponding tertiary amine. If the carbamates are kept dry, they have good thermal stability. The same precaution protects them from hydrolysis. Hydrolysis is very rapid in alkaline solutions, and slow at an acid pH. As a precaution to insure stability, the carbamates may be crystallized from solvents containing hydrogen chloride. Alternatively, acidic stabilizers such as sodium acid sulfate or hydrazine dihydrochloride may be added.

A number of the more toxic carbamates were examined for relative stability¹⁶ at a time when it appeared that stability might be a decisive factor in choice of a superior agent. The following conclusion was reached concerning thermal stability: variation in the anion of the quaternary ammonium salt results in the following order of decreasing stability: methosulfate > methiodide > methochloride. Comparing stabilities toward hydrolysis, two N,N-dimethylcarbamates were much more stable than two N-methylcarbamates (TL 1071 and TL 1217), which in turn were more stable than two N-arylcarbamates. Ultimately the two agents chosen as superior on the basis of toxicity and ease of manufacture (TL 1217 and TL 1299) were determined to be sufficiently stable for any anticipated use.

One factor with an important bearing on stability is the hygroscopic character of some of the carbamates. TL 1299 is quite hygroscopic in humid weather.² TL 1217 is not, and largely for this reason became the agent of choice. TL 1299 could be handled satisfactorily if it were needed on a large scale by controlling the humidity of the rooms in which it would be crystallized, dried, and packaged. Whereas this could be done readily on a full manufacturing scale, on the large laboratory and pilot plant scale it was much simpler to employ the nonhygroscopic methiodide, TL 1217.

13.4

TOXICOLOGY

A report has been prepared¹² which summarizes much of the toxicological work done in this country, in Britain, and in Canada on the aromatic carbamates. Tables from this report, reprinted as Table 2 of this chapter give toxicity data for the 319 aromatic carbamates and closely related compounds known to have been tested.

Aromatic carbamates prepared as part of the NDRC program were submitted to the University of Chicago Toxicity Laboratory for testing. There

they received a TL (Toxicity Laboratory) number, and were tested for subcutaneous toxicity to mice. Two to five mice were injected subcutaneously with doses of 80, 40, 20, 10, 5, and 1 mg/kg of body weight, at dilutions such that each mouse received approximately 1 per cent of its body weight in a suitable nontoxic solvent (usually water). Any compound that killed at 1.0 mg/kg was screened further and LD_{50} determinations were made for all those killing at less than 0.5 mg/kg. The data obtained are listed in Table 2, together with similar toxicity data obtained elsewhere for other aromatic carbamates.

A number of factors influencing toxicity determinations made by injection were studied carefully for the more important aromatic carbamates. One of the most important was the animal species used in testing. The leading candidates were tested in several animal species, since the object of the search was to select an agent toxic for all species. TL 1217 proved to be very toxic for all species in which it was tested. TL 1345 is the most toxic compound tested in mice, but as Table 1 shows,¹² it presents no marked superi-

intraperitoneal. (In the single comparison available for rats the intraperitoneal route was the more effective.)

3. Carbamates are relatively ineffective when administered by stomach tube, 25 to 500 times as much material being required to kill as by injection.

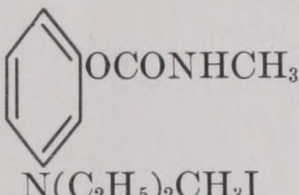
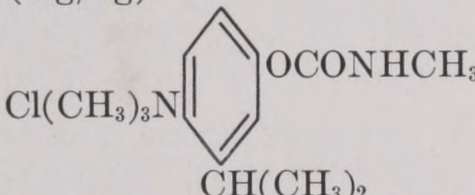
The carbamates are toxic when administered by inhalation as aerosols,¹² but do not show the extraordinary toxicity in comparison with standard chemical warfare agents which characterizes them when toxicities determined by injection are compared.

The aromatic carbamates are "quick-kill" agents capable of producing severe parasympathomimetic effects terminating in death. Death occurs rapidly, for example, in 5 to 20 minutes after subcutaneous injection in dogs. The symptoms produced are similar in all species which have been examined. They consist of salivation, evacuation of bowels and bladder, restlessness and incoordination, and fibrillary muscular movements. Respiratory movements are quickened and labored. Coma is accompanied or preceded by convulsive movements. Respiration appears to cease first, the heart beating, usually irregularly, for some moments after respiration has failed. Muscular twitching persists for some time after failure of respiration and cardiac activity.

The aromatic carbamates are powerful cholinesterase poisons, and produce marked changes in the blood. Because of medical and toxicological interest in them, their physiological mechanism of action has received considerable study. Most of this work may be located through certain leading references.^{20,21a,b,c,d,e} Atropine or atropine and pentobarbital administered intravenously have been recommended as antidotes for the carbamates.^{36,41} Antidotes can be demonstrated to be useful in animals, but must be administered quickly (at the onset of symptoms) because of the very rapid toxic action of the carbamates.

For references to toxicity assays on the carbamates in addition to the summary previously mentioned¹² see the Bibliography.^{22a,b,c,d,23,28,30,35,37-40,48,49,53}

TABLE 1. Subcutaneous toxicities of TL 1217 and TL 1345 for various species.

Species	LD_{50} (mg/kg)	
	 N(C ₂ H ₅) ₂ CH ₂ I TL 1217	 Cl(CH ₃) ₃ N CH(CH ₃) ₂ TL 1345
Mouse	0.129	0.047
Rat	ca. 0.400	0.103
Guinea pig	0.097	ca. 0.050
Rabbit	ca. 0.150	ca. 0.075
Cat	ca. 0.075	ca. 0.100
Dog	ca. 0.075	ca. 0.100
Monkey	ca. 0.200	ca. 0.150

ority over TL 1217 when other species are considered.

Other factors considered in precise toxicity determinations were the concentration of the solution injected; the strain, sex, body weight, and age of the mice used in LD_{50} determinations; and the effect of the temperature of the environment of the assay animals. A number of compounds were tested by various routes of administration, and the following conclusions were reached.¹²

1. The carbamates tested were about twice as toxic intravenously as by any other route.

2. Subcutaneous injection was more effective than

13.5 RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND TOXICITY

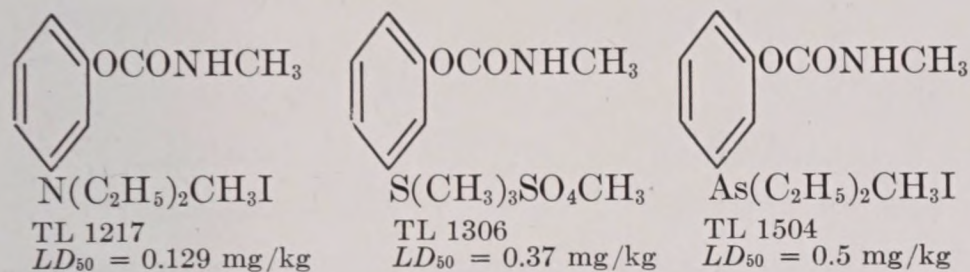
Relationships existing between chemical structure of the aromatic carbamates and their toxicity have been pointed out in some detail.¹² The following principal conclusions can be drawn from the available

toxicity data (figures cited refer to subcutaneous toxicity in mice).

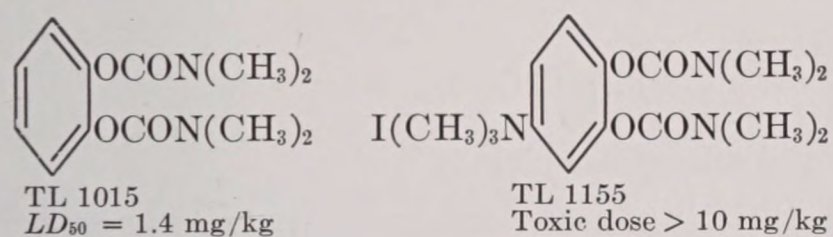
1. The most toxic compounds contain both a carbamate and a quaternary salt group.

2. The carbamate group is more intimately connected with toxicity than is the quaternary salt group. This conclusion follows from several lines of evidence:

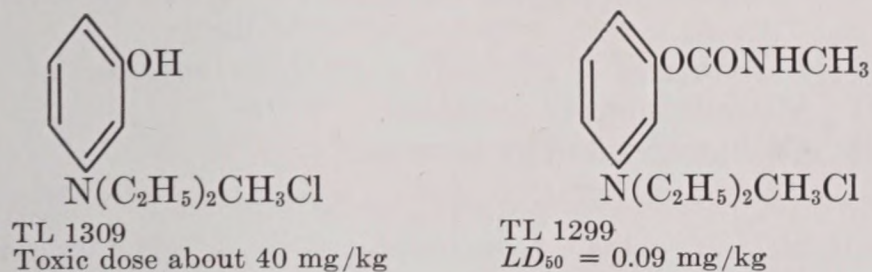
a. The quaternary ammonium group can be replaced with sulfonium or arsonium without change in order of magnitude of toxicity. For example:



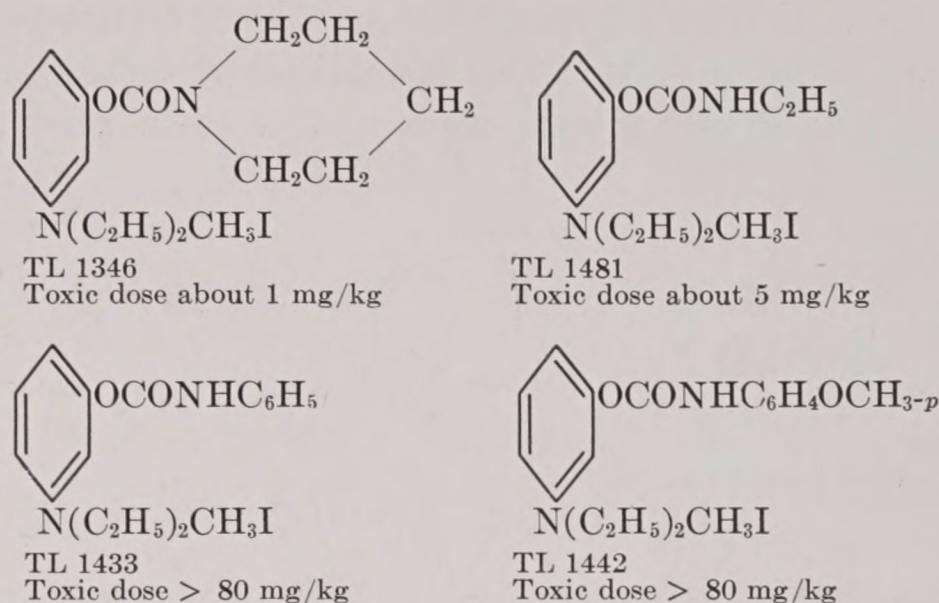
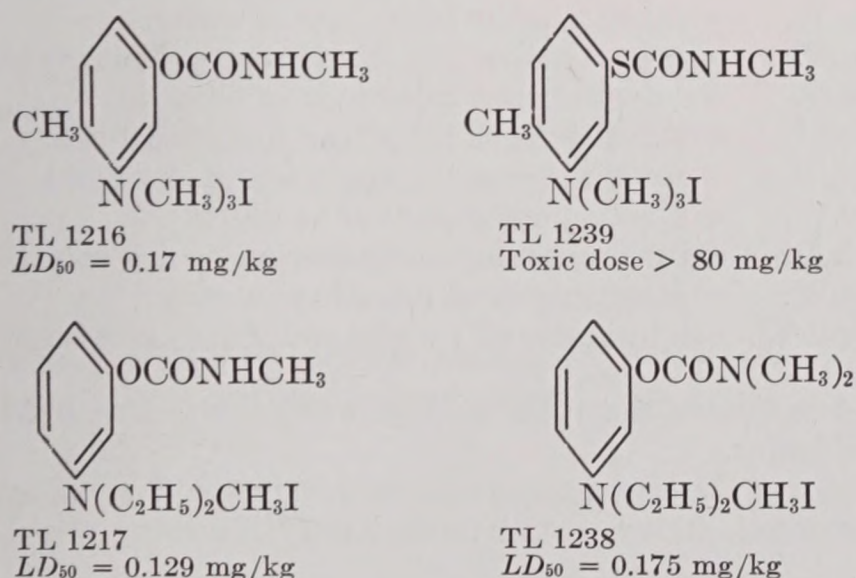
b. The *bis*-N,N-dimethylcarbamate of catechol is highly toxic even though it contains no basic group; introduction of a quaternary salt group in this compound results in diminished toxicity.



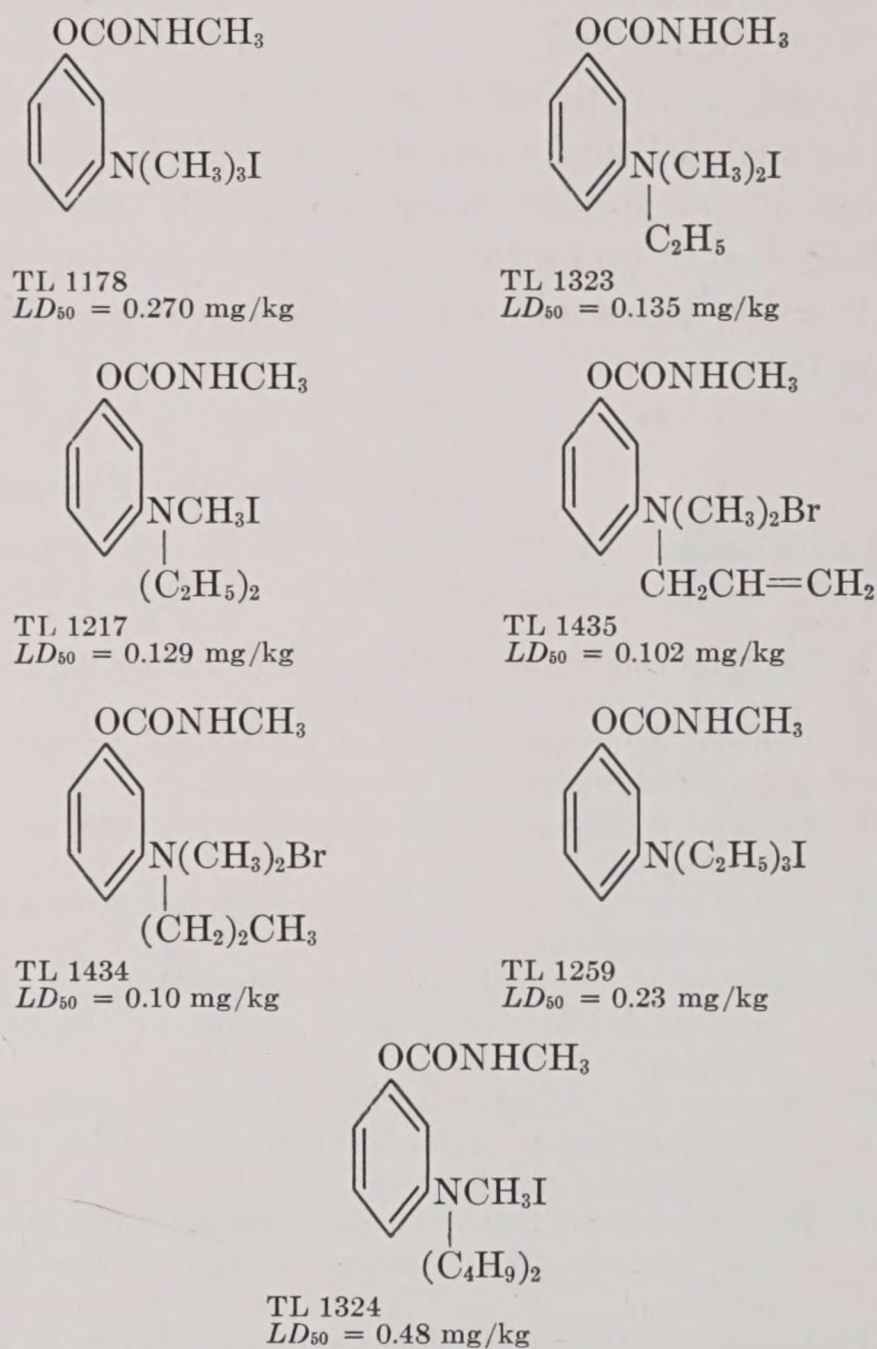
c. Quaternary salts derived from aminophenols are not very toxic, but the N-methylcarbamates derived from some of them are highly toxic.



d. Structural changes in the carbamate group in related series of compounds may produce enormous changes in their toxicity.

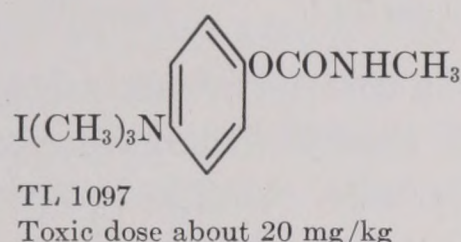
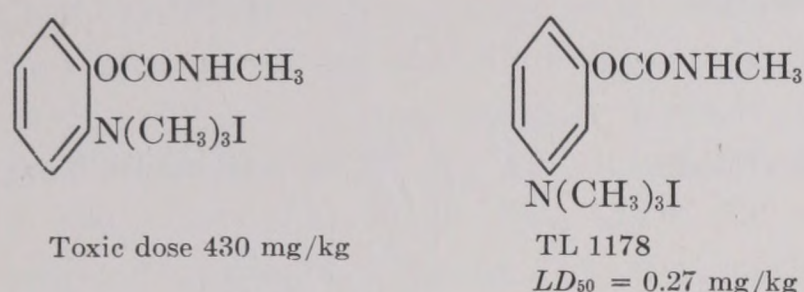


e. Changes in the quaternary salt group in a series in which the N-methylcarbamate group is kept constant produce smaller changes in toxicity.

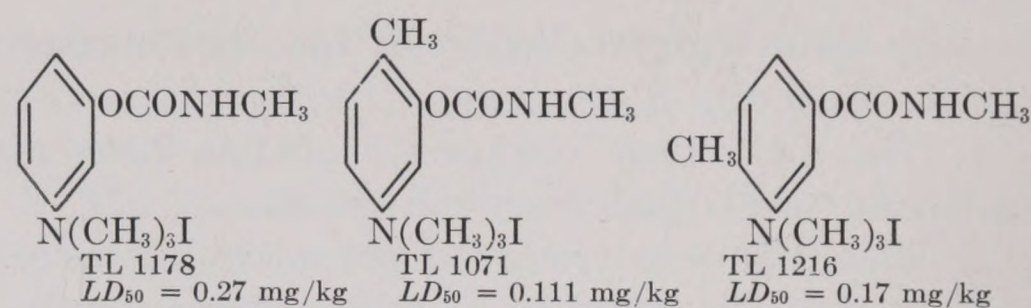


3. In general, the N-methylcarbamates are more toxic than corresponding N,N-dimethylcarbamates. Of 20 such pairs of compounds tested, the mono-methylcarbamates were more toxic in 14 cases (for some pairs they were 10 to 40 times as toxic); in the other 6 cases they were approximately equal. No other substitution on the carbamate nitrogen which was investigated led to compounds as toxic as the N-methyl and N,N-dimethyl derivatives.

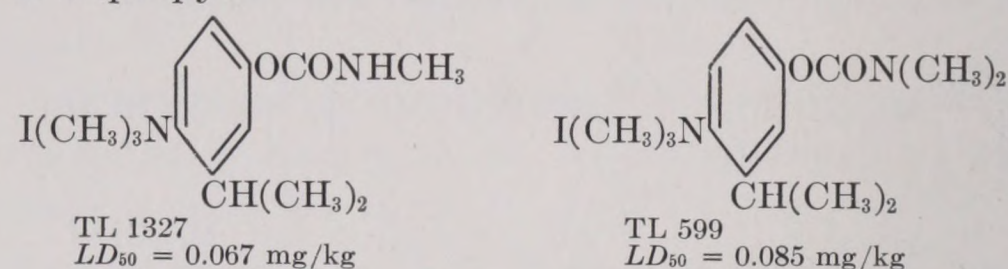
4. With few exceptions, the most toxic compounds were those in which the N-methylcarbamate and quaternary salt groups were in the meta orientation.



5. Methyl substitution in the nucleus ortho or para to the carbamate produces no great change in the toxicity of *m*-quaternary compounds, and may result in slightly more toxic substances. Similar substitution by higher alkyl groups leads to less toxic compounds.



6. The series with an alkyl substituent meta and the quaternary salt para to the carbamate group contains some extremely toxic compounds. In the most toxic homologs of this type¹¹ the alkyl group is isopropyl.



7. The toxicity of aromatic carbamates substituted by quaternary salt groups resides in the cation. Of the various salts, the chlorides have been found to be somewhat more toxic than would be calculated on a molecular weight basis. Other salts with the same cation have toxicities proportional to their molecular weights.

TABLE 2. Toxicities of aromatic carbamates and related compounds.

The following tables¹² contain the toxicity data available as of March 1945 for aromatic carbamates and closely related substances (319 in all). The tables are subdivided into 18 structural classes, as follows:

- | | |
|--|---|
| <p>I Benzene compounds with one carbamate group, and no quaternary ammonium group.</p> <p>II Benzene compounds with two carbamate groups and no other groups.</p> <p>III Benzene compounds with two carbamate groups and other groups.</p> <p>IV Benzene compounds with three carbamate groups and no other group.</p> <p>V Benzene compounds with one carbamate group and one quaternary ammonium group in the ortho position.</p> <p>VI Benzene compounds with one carbamate group and one quaternary ammonium group in the ortho position and alkyl groups.</p> <p>VII Benzene compounds with one carbamate group and one quaternary ammonium group in the meta position.</p> <p>VIII Benzene compounds with one carbamate group and one quaternary ammonium group in the meta position and other substituents.</p> <p>IX Benzene compounds with one carbamate group and one quaternary ammonium group in the para position (including thiocarbamates).</p> <p>X Benzene compounds with one carbamate group and one quaternary ammonium group in the para position and other substituents.</p> <p>XI Benzene compounds with one carbamate group and an alkyl side chain having a quaternary ammonium group.</p> <p>XII Benzene compounds with one carbamate group and two quaternary ammonium groups.</p> | <p>XIII Benzene compounds with one carbamate group and one sulfonium or arsonium group.</p> <p>XIV Carbamates of naphthalene derivatives.</p> <p>XV Carbamates of quinoline and isoquinoline derivatives.</p> <p>XVI Carbamates of aliphatic alcohol derivatives.</p> <p>XVII Miscellaneous carbamates.</p> <p>XVIII Carbamides and carbazates.</p> |
|--|---|
- The tables represent a revision of a similar review issued on June 15, 1944,¹⁹ and follow the system of classification used in the earlier summary. Entries in the column headed "Code" have the significance noted in the Glossary.
- In the column headed "Route and Solvent" the following abbreviations are used:
- | | |
|--|---|
| <p>Sc.W. = subcutaneous injections in water.</p> <p>Sc.P. = subcutaneous injections in propylene glycol.</p> <p>Sc.O. = subcutaneous injections in olive oil.</p> <p>Sc.M. = subcutaneous injections in mineral oil.</p> <p>Sc.Imp. = subcutaneous implantation of dry solid.</p> <p>Iv.W. = intravenous injection in water.</p> <p>Im.Imp. = intramuscular implantation of dry solid.</p> <p>Ip.W. = intraperitoneal injection in water.</p> <p>Oral W. = administered by stomach tube, in water.</p> | <p>pH-4 indicates that this acidity was achieved with McIlvaine's buffer.</p> <p>Whenever the room temperature during the determination was recorded, it was listed immediately following the LD₅₀ figure.</p> |
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I. Benzene compounds with one carbamate group, and no quaternary ammonium group.

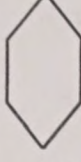
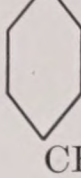
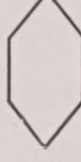
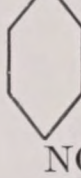
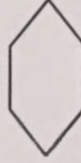
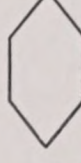
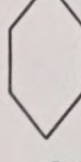
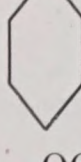
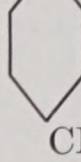
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
AR-1	Carbamic acid, N-methyl-phenyl ester	OCONHCH_3 	Iv.	Mice	>50	LD_{80}
TL-1113	Carbamic acid, N,N-dimethyl-phenyl ester	$\text{OCON}(\text{CH}_3)_2$ 	Sc.M.	Mice	80 40 20	0/2 0/2 0/2
TL-1218	Carbamthiolic acid, N-methyl- <i>p</i> -tolyl ester	SCONHCH_3 	Sc.P.	Mice	80 40 20	2/2 0/2 0/2
TL-997 AR-2	Carbamic acid, N-methyl-2-nitrophenyl ester	OCONHCH_3 	Sc.Imp. Iv.	Mice Mice	80 33	0/2 LD_{80}
TL-948	Carbamic acid, N-methyl-3-nitrophenyl ester	OCONHCH_3 	Sc.Imp.	Mice	80 40 20	0/2 0/2 0/2
TL-947	Carbamic acid, N-methyl-4-nitrophenyl ester	OCONHCH_3 	Sc.Imp.	Mice	80 40 20	0/2 0/2 0/2
TL-980	Carbamic acid, N-methyl-2-hydroxyphenyl ester	OCONHCH_3 	Sc.Imp.	Mice	80 40 20	0/2 0/2 0/2
TL-1016	Carbamic acid, N,N-dimethyl-2-hydroxyphenyl ester	$\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-979	Carbamic acid, N,N-diethyl-2-hydroxyphenyl ester	$\text{OCON}(\text{C}_2\text{H}_5)_2$ 	Sc.Imp.	Mice	80 40 20	0/2 0/2 0/2
TL-1161	Carbamic acid, N,N-dimethyl-2-allyloxyphenyl ester	$\text{OCON}(\text{CH}_3)_2$ 	Sc.O.	Mice	80 40 20	0/2 0/2 0/2
TL-1110	Carbamic acid, N,N-dimethyl-4-allyl-2-methoxyphenyl ester	$\text{OCON}(\text{CH}_3)_2$ 	Sc.M.	Mice	80 40 20	0/2 0/2 0/2

TABLE 2, Section I (Continued)

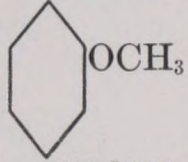
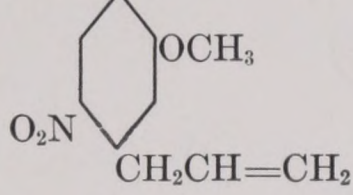
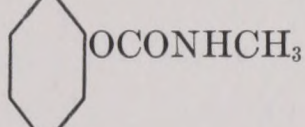
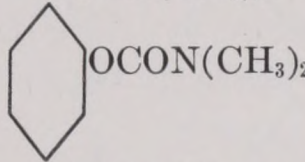
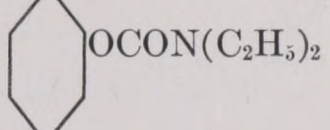
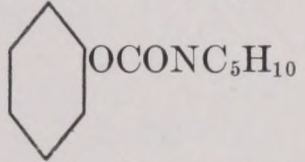
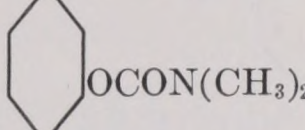
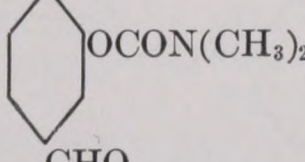
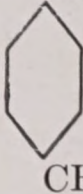
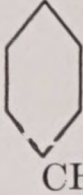
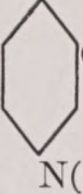
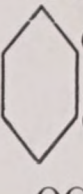
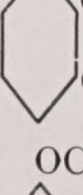
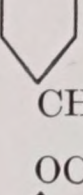
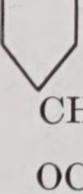
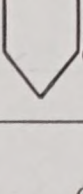
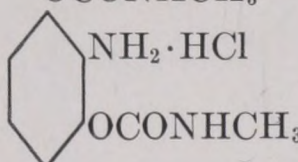
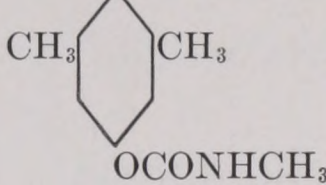
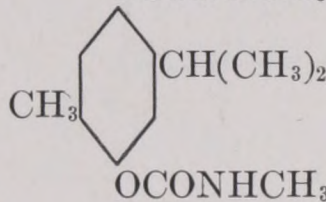
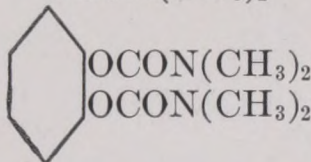
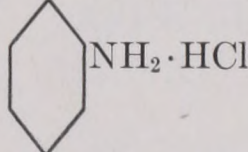
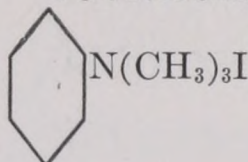
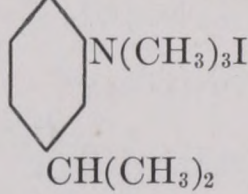
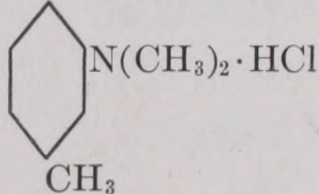
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1111	Carbamic acid, N,N-dimethyl-2-methoxy-4-propylphenyl ester	OCN(CH ₃) ₂	Sc.M.	Mice	80	0/2
					40	0/2
					20	0/2
TL-1116	Carbamic acid, N,N-dimethyl-4-allyl-2-methoxy-5-nitrophenyl ester	OCN(CH ₃) ₂	Sc.M	Mice	80	0/2
					40	0/2
					20	0/2
II. Benzene compounds with two carbamate groups and no other groups.						
TL-1015	Benzene, 1,2-bis(methylcarbamoyloxy)-	OCNHCH ₃	Sc.W.	Mice	40	2/2
					20	2/2
					10	0/2
					5	0/2
TL-978	Benzene, 1,2-bis(dimethylcarbamoyloxy)-	OCN(CH ₃) ₂	Sc.P.	Mice	1.4	LD ₅₀
						
TL-1118	Benzene, 1,2-bis(diethylcarbamoyloxy)-	OCN(C ₂ H ₅) ₂	Sc.P	Mice	80	0/2
					40	0/2
					20	0/2
TL-1119	Benzene, 1,2-bis(N-pentamethylenecarbamoyloxy)-	OCNC ₅ H ₁₀	Sc.M	Mice	80	0/2
					40	0/2
					20	0/2
TL-1112	Benzene, 1,3-bis(dimethylcarbamoyloxy)-	OCN(CH ₃) ₂	Sc.W.	Mice	80	0/2
					40	0/2
					20	0/2
TL-1114	Benzene, 1,4-bis(dimethylcarbamoyloxy)-	OCN(CH ₃) ₂	Sc.P	Mice	80	0/2
					40	0/2
					20	0/2
TL-1348	Benzene, 1,4-bis(methylcarbamoyloxy)-	OCN(CH ₃) ₂	Sc.P.	Mice	80	0/2
		OCNHCH ₃			40	0/2
					20	0/2
III. Benzene compounds with two carbamate groups and other groups.						
TL-1117	Benzaldehyde, 3,4-bis(dimethylcarbamoyloxy)-	OCN(CH ₃) ₂	Sc.P.	Mice	80	0/2
					40	0/2
					20	0/2

TABLE 2, Section III (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1157	Benzyl alcohol, 3,4- <i>bis</i> (dimethylcarbamyloxy)-	$\text{OCON}(\text{CH}_3)_2$  CH_2OH	Sc.W.	Mice	10 5 1	1/2 0/2 0/2
TL-1160	Dimethylamine, N-[3,4- <i>bis</i> (dimethylcarbamyloxy)benzyl] hydrochloride	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-981	Benzene, 1,2- <i>bis</i> (dimethylcarbamyloxy)-4-nitro-	$\text{OCON}(\text{CH}_3)_2$  NO_2	Sc.Imp. Sc.O.	Mice	80 80 40	0/2 0/2 0/2
TL-1017	Benzene, 1,2- <i>bis</i> (dimethylcarbamyloxy)-4-amino-	$\text{OCON}(\text{CH}_3)_2$  NH_2	Sc. N/10 Ac.	Mice	40 20 10 5	2/2 2/2 2/2 0/2
TL-1155	Benzene, 4-(dimethylamino)-1,2- <i>bis</i> (dimethylcarbamyloxy)-, methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 1 0.5	0/2 0/2 0/2 0/2
TL-1159	Benzene, 1,2- <i>bis</i> (dimethylcarbamyloxy)-3-allyl-	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{CH}=\text{CH}_2$	Sc.O.	Mice	10 5 1 0.5	0/2 0/2 0/2 0/2
TL-1158	Benzene, 1,2- <i>bis</i> (dimethylcarbamyloxy)-3-propyl-	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{CH}_2\text{CH}_3$	Sc.O.	Mice	10 5 1 0.5	0/2 0/2 0/2 0/2
TL-1162	Benzene, 1,2- <i>bis</i> (dimethylcarbamyloxy)-4-allyl-	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{CH}=\text{CH}_2$	Sc.O.	Mice	10 5 1 0.5	0/2 0/2 0/2 0/2
TL-1156	Benzene, 1,2- <i>bis</i> (dimethylcarbamyloxy)-4-propyl-	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{CH}_2\text{CH}_3$	Sc.M.	Mice	10 5 1 0.5	0/2 0/2 0/2 0/2
TL-1086	Benzene, 1,3- <i>bis</i> (N-methylcarbamyloxy)-2-nitro-	OCONHCH_3  NO_2	Sc.P.	Mice	40 20	0/2 0/2

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TABLE 2, Section III (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1129	Benzene, 1,3- <i>bis</i> (N-methylcarbamoyloxy)-2-amino hydrochloride		Sc.W.	Mice	80	0/2
					40	0/2
					20	0/2
TL-1349	Benzene, 1,4- <i>bis</i> (methylcarbamoyloxy)-2,6-dimethyl-		Sc.P.	Mice	80	0/2
					40	0/2
					20	0/2
TL-1350	Benzene, 1,4- <i>bis</i> (methylcarbamoyloxy)-2-isopropyl-5-methyl-		Sc.P.	Mice	80	2/2
					40	1/2
					20	1/2
					10	0/2
					5	0/2
IV. Benzene compounds with three carbamate groups and no other group.						
TL-1115	Benzene, 1,2,3- <i>tris</i> (dimethylcarbamoyloxy)-		Sc.W.	Mice	40	2/2
					20	2/2
					10	0/2
					5	0/2
V. Benzene compounds with one carbamate group and one quaternary ammonium group in the ortho position.						
TL-963	Carbamic acid, N-methyl-2-aminophenyl ester hydrochloride		Sc.W.	Mice	80	0/2
					40	0/2
					20	0/2
T-(?)	Carbamic acid, N-methyl-2-dimethylaminophenyl ester methiodide		Sc.	Mice	430	LD ₅₀
VI. Benzene compounds with one carbamate group and one quaternary ammonium group in the ortho position and alkyl groups.						
TL-1488	Carbamic acid, N-methyl-2-dimethylamino-4-isopropylphenyl ester methiodide		Sc.W.	Mice	80	0/2
					40	0/2
					20	0/2
SB-13	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-methylphenyl ester hydrochloride		Sc.	Mice	Approx. 200	LD ₅₀

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TABLE 2, Section VI (Continued)

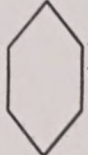
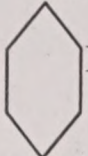
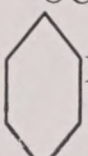
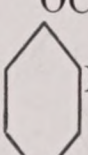
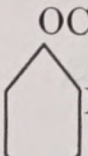
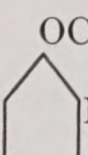
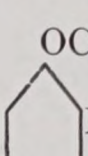
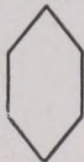
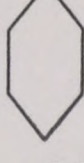
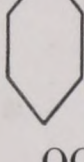
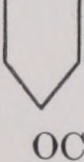
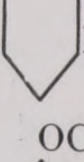
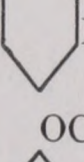
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
SB-14	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-methylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_3\text{I}$ CH_3	Sc.	Mice	2.0	LD_{50}
SB-15	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-ethylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$ C_2H_5	Sc.	Mice	27	LD_{50}
SB-16	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-ethylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_3\text{I}$ C_2H_5	Sc.	Mice	1.25	LD_{50}
SB-17	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-isopropylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$ $\text{CH}(\text{CH}_3)_2$	Sc.	Mice	>400	LD_{50}
SB-18	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-isopropylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_3\text{I}$ $\text{CH}(\text{CH}_3)_2$	Sc.	Mice	4.8	LD_{50}
SB-19	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-tert butylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$ $\text{C}(\text{CH}_3)_3$	Sc.	Mice	>500	LD_{50}
SB-20	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-tert butylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_3\text{I}$ $\text{C}(\text{CH}_3)_3$	Sc.	Mice	13.5	LD_{50}
SB-21	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-tert amylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$ $\text{C}_2\text{H}_5\text{C}(\text{CH}_3)_2$	Sc.	Mice	>500	LD_{50}
SB-22	Carbamic acid, N,N-dimethyl-2-dimethylamino-4-tert amylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_3\text{I}$ $\text{C}_2\text{H}_5\text{C}(\text{CH}_3)_2$	Sc.	Mice	12	LD_{50}

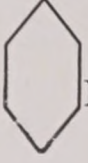
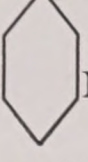
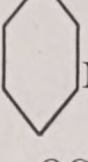
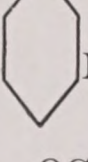
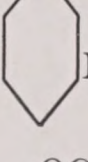
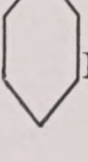
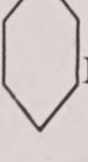
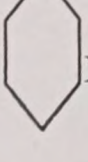
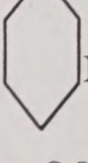
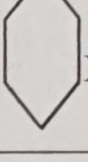
TABLE 2 (Continued)

VII. Benzene compounds with one carbamate group and one quaternary ammonium group in the meta position.

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1309	Phenol, 3-(diethylamino)-methochloride	OH	Sc.W.	Mice	80	2/2
					40	2/2
		N(C ₂ H ₅) ₂ CH ₃ Cl			20	0/2
					10	0/2
T-1122	Carbamic acid, 3-dimethylaminophenyl ester methiodide	OCONH ₂ 	Sc.	Mice	37	LD ₅₀
AR-11	Carbamic acid, 3-dimethylaminophenyl ester methosulfate	OCONH ₂ 	Iv.	Mice	0.7	LD ₈₀
TL-946	Carbamic acid, N-methyl-3-aminophenyl ester hydrochloride	OCONHCH ₃	Sc.W.	Mice	80	0/2
					40	0/2
		NH ₂ ·HCl			20	0/2
AR-12	Carbamic acid, N-methyl-3-dimethylaminophenyl ester hydrochloride	OCONHCH ₃ 	Iv.	Mice	15	LD ₈₀
T-1152	Carbamic acid, N-methyl-3-dimethylaminophenyl ester methiodide	OCONHCH ₃	Sc.	Mice	0.44	LD ₅₀
			Sc.	Rabbit	0.26	
		N(CH ₃) ₃ I	Sc.	Mice	30	
TL-1178	Carbamic acid, N-methyl-3-dimethylaminophenyl ester methiodide	OCONHCH ₃	Sc.W.	Mice	0.27	LD ₅₀
			Iv.W.	Mice	0.115	LD ₅₀
			(See p. 219)			
TL-1226	Carbamic acid, N-methyl-3-dimethylaminophenyl ester methochloride	OCONHCH ₃	Sc.W.	Mice	0.140	LD ₅₀
			Iv.W.	Mice	0.070	LD ₅₀
T-1690	Carbamic acid, N-methyl-3-dimethylaminophenyl ester methochloride	OCONHCH ₃ 	Sc.	Mice	0.27	LD ₅₀
AR-13	Carbamic acid, N-methyl-3-dimethylaminophenyl ester methosulfate	OCONHCH ₃ 	Iv.	Mice	0.1	LD ₈₀
TL-1323	Carbamic acid, N-methyl-3-dimethylaminophenyl ester ethiodide	OCONHCH ₃	Sc.W.	Mice	0.135	LD ₅₀
T-1194			Sc.	Mice	0.38	LD ₅₀
			Sc.	Rabbit	0.13	LD ₅₀

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TABLE 2, Section VII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
AR-14	Carbamic acid, N-methyl-3-ethylmethylaminophenyl ester methobromide	OCONHCH_3  $\text{N}(\text{CH}_3)_2\text{C}_2\text{H}_5\text{Br}$	Iv.	Mice	0.15	LD_{80}
TL-1434	Carbamic acid, N-methyl-3-dimethylaminophenyl ester propyl bromide	OCONHCH_3  $\text{N}(\text{CH}_3)_2\text{CH}_2\text{CH}_2\text{CH}_3\text{Br}$	Sc.W.	Mice (See p. 219)	0.100 (78 F)	LD_{50}
TL-1435	Carbamic acid, N-methyl-3-dimethylaminophenyl ester allyl bromide	OCONHCH_3  $\text{N}(\text{CH}_3)_2\text{CH}_2\text{CH}=\text{CH}_2\text{Br}$	Sc.W.	Mice (See p. 219)	0.102 (82 F)	LD_{50}
TL-1324	Carbamic acid, N-methyl-3-dibutylaminophenyl ester methiodide	OCONHCH_3  $\text{N}(\text{C}_4\text{H}_9)_2\text{CH}_3\text{I}$	Sc.W.	Mice	0.48	LD_{50}
AR-15	Carbamic acid, N-methyl-3-diethylaminophenyl ester hydrochloride	OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_2 \cdot \text{HCl}$	Iv.	Mice	5.0	LD_{50}
TL-1217	Carbamic acid, N-methyl-3-diethylaminophenyl ester methiodide	OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$	Sc.W. Sc.W. Sc.W. pH4 Sc.W.	Mice Mice Mice G. pig (See p. 219)	0.122* 0.129 0.135 0.097	LD_{50} LD_{50} LD_{50} LD_{50}
T-1123 AR-16			Sc. Iv. Sc.	Mice Mice Mice	0.29 0.1 0.13	LD_{50} LD_{80} LD_{50}
TL-1299 Prep. 1 Prep. 2 Prep. 3 Prep. 3	Carbamic acid, N-methyl-3-diethylaminophenyl ester methochloride	OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{Cl}$	Sc.W. Sc.W. Sc.W. Sc.W.	Mice Mice Mice Mice (See p. 220)	0.090† 0.097 0.105‡ 0.095§	LD_{50} LD_{50} LD_{50} LD_{50}
TL-1317	Carbamic acid, N-methyl-3-diethylaminophenyl ester methosulfate	OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_2(\text{CH}_3)_2\text{SO}_4$	Sc.W. pH4 Sc.W. Sc.W. Sc.W.	Mice Mice Mice Mice (See p. 220)	0.100 0.114 0.107 0.102	LD_{50} LD_{50} LD_{50} LD_{50}
TL-1259	Carbamic acid, N-methyl-3-diethylaminophenyl ester ethiodide	OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_3\text{I}$	Sc.W.	Mice	0.23	LD_{50}
AR-31	Carbamic acid, N,N-dimethyl-3-dimethylaminophenyl ester acid tartrate	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2(-\text{CHOHCOOH})_2$	Iv.	Mice	60	LD_{80}

* At 85 F.

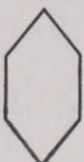
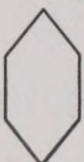
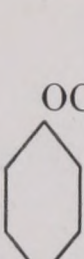
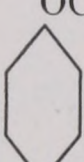
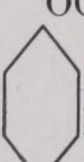
† At 75 F.

‡ At 77 F.

§ At 76 F.

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TABLE 2, Section VII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1321 SB-24	Carbamic acid, N,N-dimethyl-3-dimethylaminophenyl ester methiodide		Sc.W. Sc.	Mice Mice	0.475 0.55	LD_{50} LD_{50}
AR-32 SB-23 TL-1394	Carbamic acid, N,N-dimethyl-3-dimethylaminophenyl ester methosulfate (Prostigmine)		Iv. Sc.	Mice Mice (See p. 220)	0.5 0.45	LD_{80} LD_{50}
TL-1238 Prep. 1	Carbamic acid, N,N-dimethyl-3-diethylaminophenyl ester methiodide		Sc.W.	Mice	80 40 20 10	2/2 2/2 0/2 0/2
Prep. 2 Prep. 3 Prep. 3			Sc.W. Sc.W. Iv.W.	Mice Mice Mice (See p. 220)	0.125* 0.175† 0.089†	LD_{50} LD_{50} LD_{50}
TL-1422	Carbamic acid, N,N-dimethyl-3-diethylaminophenyl ester methochloride		Sc.W. Sc.W. Sc.W.	Mice Mice Mice (See p. 220)	0.058‡ 0.108§ 0.100	LD_{50} LD_{50} LD_{50}
TL-1481	Carbamic acid, N-ethyl-3-diethylaminophenyl ester methiodide		Sc.W.	Mice	10.0 5.0 2.5 1.0	2/2 2/2 0/2 0/2
AR-21	Carbamic acid, N-ethyl-3-dimethylaminophenyl ester methosulfate		Iv.	Mice	1.0	LD_{80}
AR-36	Carbamic acid, N-ethyl-N-methyl-3-dimethylaminophenyl ester methosulfate		Iv.	Mice	3.5	LD_{80}
AR-33	Carbamic acid, N,N-diethyl-3-dimethylaminophenyl ester methosulfate		Iv.	Mice	8	LD_{80}
AR-19	Carbamic acid, N-allyl-3-dimethylaminophenyl ester hydrochloride		Iv.	Mice	150	LD_{80}
AR-34	Carbamic acid, N,N-diallyl-3-dimethylaminophenyl ester methiodide		Iv.	Mice	10	LD_{80}

* At 83 F.

† At 75 F.

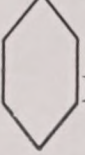
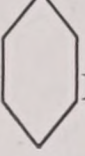
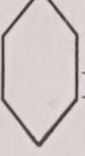
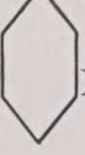
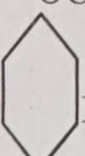
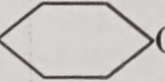
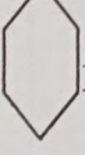
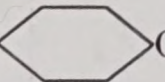
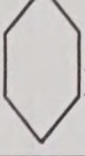
‡ At 80 F.

§ At 71 F.

|| At 73 F.

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TABLE 2, Section VII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
AR-20	Carbamic acid, N-allyl-3-dimethylaminophenyl ester methosulfate	$\text{OCONHCH}_2\text{CH}=\text{CH}_2$  $\text{N}(\text{CH}_3)_3\text{SO}_4\text{CH}_3$	Iv.	Mice	0.75	LD_{80}
AR-24	Carbamic acid, N-phenyl-3-dimethylaminophenyl ester hydrochloride	$\text{OCONHC}_6\text{H}_5$  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	Iv.	Mice	25	LD_{80}
AR-25	Carbamic acid, N-phenyl-3-dimethylaminophenyl ester methosulfate	$\text{OCONHC}_6\text{H}_5$  $\text{N}(\text{CH}_3)_3\text{SO}_4\text{CH}_3$	Iv.	Mice	2	LD_{80}
AR-22	Carbamic acid, N-benzyl-3-dimethylaminophenyl ester hydrochloride	$\text{OCONHCH}_2\text{C}_6\text{H}_5$  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	Iv.	Mice	50	LD_{80}
AR-23	Carbamic acid, N-benzyl-3-dimethylaminophenyl ester methosulfate	$\text{OCONHCH}_2\text{C}_6\text{H}_5$  $\text{N}(\text{CH}_3)_3\text{SO}_4\text{CH}_3$	Iv.	Mice	0.1	LD_{80}
T-1125	Carbamic acid, N-benzyl-3-dimethylaminophenyl ester methiodide	$\text{OCONHCH}_2\text{C}_6\text{H}_5$  $\text{N}(\text{CH}_3)_3\text{I}$	Sc. Sc.	Mice Rabbit	0.35 0.20	LD_{50} LD_{50}
TL-1308	Carbamic acid, N,N-pentamethylene-3-dimethylaminophenyl ester methiodide	$\text{OCONC}_5\text{H}_{10}$  $\text{N}(\text{CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 2.5	2/2 2/2 0/2
AR-35	Carbamic acid, N,N-pentamethylene-3-dimethylaminophenyl ester methosulfate	$\text{OCONC}_5\text{H}_{10}$  $\text{N}(\text{CH}_3)_3\text{SO}_4\text{CH}_3$	Iv.	Mice	6	LD_{80}
TL-1346	Carbamic acid, N,N-pentamethylene-3-diethylaminophenyl ester methiodide	$\text{OCONC}_5\text{H}_{10}$  $\text{N}(\text{C}_2\text{H}_5)_2$ CH_3I	Sc.W.	Mice	5 1 0.2 0.1	5/5 3/5 0/5 0/5
T-1207	Carbamic acid, N-(4-methoxyphenyl)-3-dimethylaminophenyl ester methiodide	OCONH  OCH_3  $\text{N}(\text{CH}_3)_3\text{I}$	Sc.	Mice	0.24	LD_{50}
TL-1442	Carbamic acid, N-(4-methoxyphenyl)-3-diethylaminophenyl ester methiodide	OCONH  OCH_3  $\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$	Sc.P.	Mice	80 40 20	0/2 0/2 0/2

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TABLE 2, Section VII (Continued)

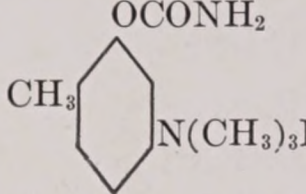
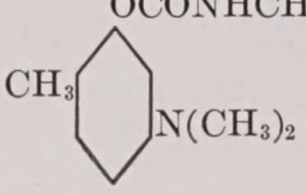
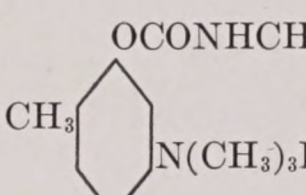
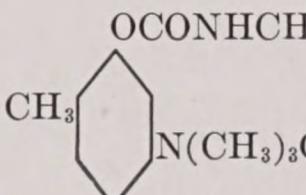
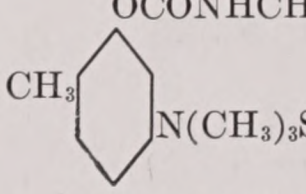
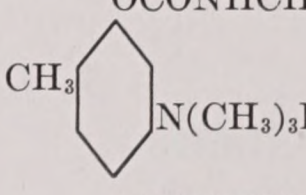
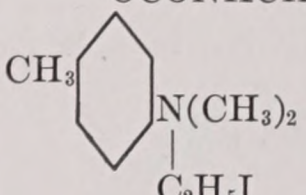
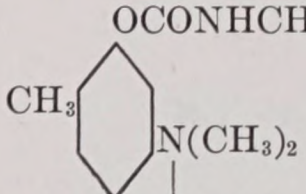
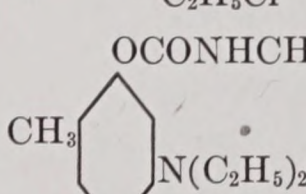
Code	Route and solvent	Species	Effects (at various doses)				
			0.10	0.125	0.25	0.5	1.0
TL-1178	Sc.W.		...	...	0/2	1/2	2/2
		Rat	0/2	...	2/2	2/2	2/2
		Rabbit	...	0/2	2/2	2/2	2/2
		G. pig	0/1	0/1	0/1	0/2	1/2
		Dog	0/2	...	0/2	2/2	...
		Cat					
TL-1434	Sc.W.		0.025	0.050	0.100	0.200	
		Rat	...	...	0/2	2/2	...
		Rabbit	...	0/2	1/2	2/2	...
		G. pig	...	0/2	1/2	2/2	...
		Cat	0/2	2/2	2/2	...	...
		Dog	0/2	1/2	1/2	2/2	...
TL-1435	Sc.W.		0.050	0.100	0.200		
		Rat	...	0/2	2/2	...	...
		Rabbit	...	0/2	2/2	...	...
		G. pig	0/2	2/2	2/2	...	...
		Dog	...	0/2	2/2	...	...
TL-1217	Sc.W.		0.05	0.1	0.2	0.3	0.4
		Rat	...	0/2	0/2	0/7	6/7
		Rabbit	...	0/2	2/2	...	...
		G. pig	...	0/2	2/2	...	...
		Dog	1/2	2/2	2/2	...	...
		Cat	0/2	2/2	2/2	...	...
		Sheep	...	0/2	3/3	...	...
		Goat	...	...	0/2	2/5	2/3
		Monkey	...	0/2	2/3	...	...

Code	Route and solvent	Species	Effects (at various doses)						
			0.025	0.05	0.1	0.2	0.3		
TL-1299 (2nd sample)	Im.Imp.		...	...	0/1	0/1	1/1	...	...
		Goat	0/2	0/4	1/1	1/1	...	...	...
		Monkey	...	0/3	1/3	8/10	...	...	...
		Dog							
TL-1317	Sc.W.		0.05	0.1	0.2	0.3			
		Rat	...	0/2	3/5	2/2	...	...	...
		G. pig	0/2	1/5	5/5	...	...	...	...
		Rabbit	0/2	1/2	2/2	...	...	...	...
		Cat	...	0/2	2/2	...	...	...	...
TL-1394	Sc.W.		0.2	0.5	1.0	1.5			
		Rat	...	0/2	1/2	2/2	...	...	...
		Rabbit	...	0/2	2/2	2/2	...	...	...
		G. pig	0/2	2/2	2/2	2/2	...	...	...
		Dog	0/2	1/2	1/2	...	...	...	...
		Cat	0/2	1/2	2/2	...	...	...	...
TL-1238	Sc.W.		0.05	0.1	0.15	0.2	0.3	0.4	1.0
		Rat	...	...	0/2	0/2	0/2	1/2	2/2
		Rabbit	...	0/2	1/2	2/2	...	...	...
		G. pig	0/2	3/6	...	2/2	...	...	...
		Cat	...	0/2	...	2/2	...	...	...
		Dog	...	...	...	0/2	1/2	2/2	...
TL-1422	Sc.W.		0.05	0.10					
		Rabbit	0/2	2/2	...	...	...	...	...
		Dog	0/2	2/2	...	...	...	...	...

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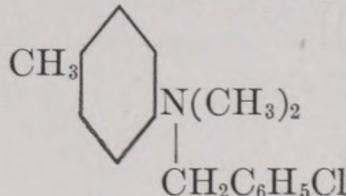
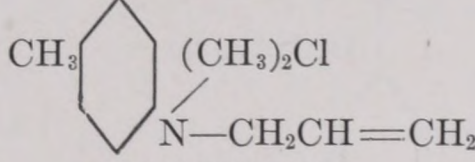
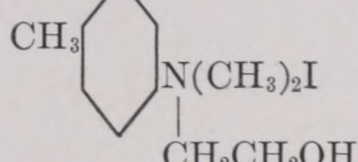
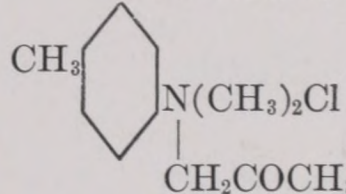
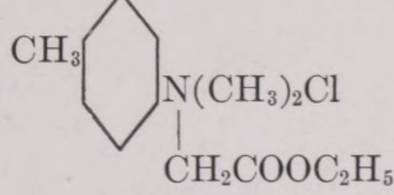
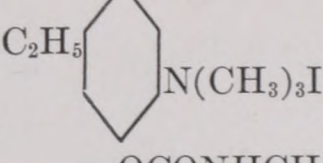
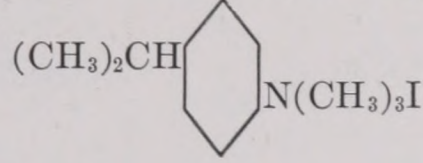
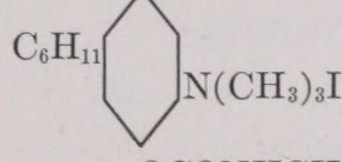
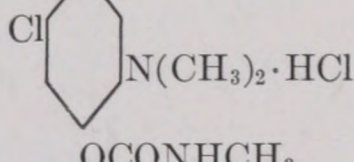
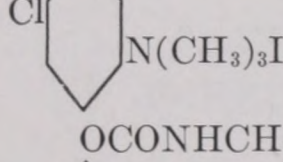
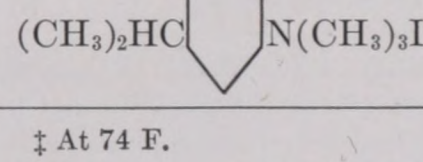
TABLE 2 (Continued)

VIII. Benzene compounds with one carbamate group and one quaternary ammonium group in the meta position and other substituents.

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1256	Carbamic acid, 2-methyl-5-dimethylaminophenyl ester methiodide		Sc.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-1184	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester		Sc.P.	Mice	80 40 20	1/2 0/2 0/2
			Sc.W. pH3	Mice	80 40 20	5/5 5/5 1/5
T-1708 TL-1071	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester methiodide		Sc. Sc.W. Sc.W. pH4 Sc.W. pH4 Sc.W. pH4	Mice Mice Mice Mice Mice	0.1-0.12 0.115 0.108 0.107 0.102	LD ₅₀ LD ₅₀ LD ₅₀ LD ₅₀ LD ₅₀
TL-1236 Prep. 1 Prep. 2 Prep. 2 Prep. 2 Prep. 2 Prep. 2 Prep. 2	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester methochloride		Sc.W. Sc.W. Ip.W. Sc.W. Ip.W. Oral W. Sc.W.	Mice Mice Mice Rats Rats Rats Dogs	0.075 0.064 0.088 0.100 0.078 2.5 2.0	LD ₅₀ LD ₅₀ LD ₅₀ LD ₅₀ LD ₅₀ LD ₅₀ 4/10
					1.0	0/3
Prep. 2 Prep. 3 Prep. 3			Im.Imp. Sc.W. Iv.W.	Monkeys Mice Mice	0.050 0.070 0.035	1/4 LD ₅₀ LD ₅₀
TL-1185	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester methosulfate		Sc.W.	Mice (See p. 224)	0.110	LD ₅₀
TL-1186	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester methosulfuric acid		Sc.W.	Mice (See p. 224)	0.103	LD ₅₀
TL-1340	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester ethiodide		Sc.W.	Mice	0.090	LD ₅₀
TL-1339	Carbamic acid, N-methyl-2-methyl-5-dimethylaminophenyl ester ethochloride		Sc.W.	Mice	0.075*	LD ₅₀
TL-1257 T-1739	Carbamic acid, N-methyl-2-methyl-5-diethylaminophenyl ester methiodide		Sc.W. ?	Mice Mice	0.125 0.2	LD ₅₀ LD ₅₀

* At 85 F.

TABLE 2, Section VIII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1262	Carbamic acid, N-methyl-2-methyl-5-(N-benzyl-N-methylamino)phenyl ester methochloride		Sc.W.	Mice	10	2/2
					5	2/2
					2.5	0/2
					2	0/2
TL-1261	Carbamic acid, N-methyl-2-methyl-5-(N-allyl-N-methylamino)phenyl ester methochloride		Sc.W.	Mice	0.077	LD ₅₀
TL-1511	Carbamic acid, N-methyl-2-methyl-5-dimethylamino-phenyl ester β-hydroxyethiodide		Sc.W.	Mice	0.118*	LD ₅₀
TL-1512	Carbamic acid, N-methyl-2-methyl-5-dimethylamino-phenyl ester acetonylchloride		Sc.W.	Mice	0.056†	LD ₅₀
TL-1513	Carbamic acid, N-methyl-2-methyl-5-dimethylamino-phenyl ester carbethoxymethochloride		Sc.W.	Mice	0.165‡	LD ₅₀
T-1722	Carbamic acid, N-methyl-3-dimethylamino-6-ethyl-phenyl ester methiodide		(In saline) (In buffer solution)	Mice Mice	0.75 1.36	LD ₅₀ LD ₅₀
T-1709	Carbamic acid, N-methyl-5-dimethylamino-2-isopropyl-phenyl ester methiodide		?	Mice	250-300	LD ₅₀
TL-1501			(In buffer solution)	Mice	125	LD ₅₀
			Sc.W.	Mice	80	0/2
					40	0/2
					20	0/2
T-1778	Carbamic acid, N-methyl-2-cyclohexyl-5-dimethylamino-phenyl ester methiodide		?	Mice	175	LD ₅₀
T-1842	Carbamic acid, N-methyl-2-chloro-5-dimethylamino-phenyl ester hydrochloride		?	Mice	45	LD ₅₀
T-1800	Carbamic acid, N-methyl-2-chloro-5-dimethylamino-phenyl ester methiodide		?	Mice	4	LD ₅₀
TL-1523	Carbamic acid, N-methyl-3-isopropyl-5-dimethylamino-phenyl ester methiodide		Sc.W.	Mice	0.120 (78 F)	LD ₅₀

* At 76 F.

† At 73 F.

‡ At 74 F.

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TABLE 2, Section VIII (Continued)

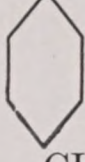
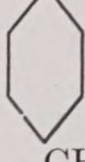
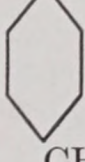
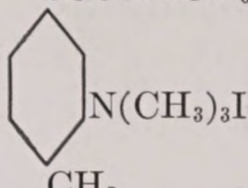
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1768	Carbamic acid, N-methyl-3-dimethylamino-4-methylphenyl ester hydrochloride	OCONHCH_3  $\text{N(CH}_3)_2 \cdot \text{HCl}$ CH_3	?	Mice	10-15	LD_{50}
TL-1187	Carbamic acid, N-methyl-4-methyl-3-dimethylamino-phenyl ester	OCONHCH_3  $\text{N(CH}_3)_2$ CH_3	Sc.P.	Mice	80	2/2
					40	2/2
					20	2/2
					10	0/2
			Sc.W. pH3	Mice	5	0/2
					80	5/5
					40	5/5
					20	5/5
TL-1216	Carbamic acid, N-methyl-4-methyl-3-dimethylamino-phenyl ester methiodide	OCONHCH_3  $\text{N(CH}_3)_3\text{I}$ CH_3	Sc.W.	Mice	0.170	LD_{50}
TL-1453	Carbamic acid, N-methyl-4-methyl-3-dimethylamino-phenyl ester methochloride	OCONHCH_3  $\text{N(CH}_3)_3\text{Cl}$ CH_3	Sc.W.	Mice	0.130 (75 F)	LD_{50}
TL-1429	Carbamic acid, N-methyl-4-methyl-3-dimethylamino-phenyl ester ethiodide	OCONHCH_3  $\text{N(CH}_3)_2\text{C}_2\text{H}_5\text{I}$ CH_3	Sc.W.	Mice	0.155 (72 F)	LD_{50}
TL-1188	Carbamic acid, N-methyl-4-methyl-3-dimethylamino-phenyl ester methosulfate	OCONHCH_3  $\text{N(CH}_3)_3\text{SO}_4\text{CH}_3$ CH_3	Sc.W.	Mice (See p. 224)	0.200	LD_{50}
TL-1354	Carbamic acid, N-methyl-4-methyl-3-dimethylamino-phenyl ester allyl bromide	OCONHCH_3  $\text{N(CH}_3)_2\text{CH}_2\text{CH}=\text{CH}_2\text{Br}$ CH_3	Sc.W.	Mice	0.095	LD_{50}
TL-1338	Carbamic acid, N-methyl-4-methyl-3-methylbenzylaminophenyl ester methobromide	OCONHCH_3  $\text{NCH}_2\text{C}_6\text{H}_5$ CH_3	Sc.W.	Mice	10	3/3
					5	2/3
					1	0/3
					0.5	0/3
T-1769	Carbamic acid, N-methyl-3-dimethylamino-4-isopropylphenyl ester hydrochloride	OCONHCH_3  $\text{N(CH}_3)_2 \cdot \text{HCl}$ $\text{CH(CH}_3)_2$	(In buffer solution)	Mice	70	LD_{50}

TABLE 2, Section VIII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1502	Carbamic acid, N-methyl-3-dimethylamino-4-isopropylphenyl ester methiodide	OCONHCH_3 	Sc.W	Mice	0.51 (75 F)	LD_{50}
T-1721				Mice	1	
T-1770	Carbamic acid, N-methyl-2,4-dimethyl-5-dimethylamino-phenyl ester hydrochloride	$\text{CH(CH}_3)_2$ OCONHCH_3 	?	Mice	10	LD_{50}
T-1767	Carbamic acid, N-methyl-2,4-dimethyl-5-dimethylamino-phenyl ester methiodide	CH_3 OCONHCH_3 	?	Mice	0.1	LD_{50}
T-1740	Carbamic acid, N-methyl-3-dimethylamino-5-methylphenyl ester methiodide	CH_3 OCONHCH_3 	?	Mice	0.17	LD_{50}
T-1741	Carbamic acid, N-methyl-3-dimethylamino-4-ethylphenyl ester methiodide	OCONHCH_3 	?	Mice	0.4	LD_{50}
TL-1237	Carbamic acid, N,N-dimethyl-2-methyl-5-dimethylamino-phenyl ester methiodide	C_2H_5 $\text{OCON(CH}_3)_2$ 	Sc.W.	Mice	10 5 2.5 2.0 1.0	2/2 2/2 2/2 0/5 0/5
TL-1423	Carbamic acid, N, N-dimethyl-2-methyl-5-diethylamino-phenyl ester methiodide	$\text{OCON(CH}_3)_2$ 	Sc.W.	Mice	10 5 0.5 0.25	2/2 2/4 0/5 0/5
TL-1325	Carbamic acid, N,N-dimethyl-4-methyl-3-dimethylamino-phenyl ester methiodide	$\text{OCON(CH}_3)_2$ 	Sc.W.	Mice	10 5 1 0.5 0.2	2/2 2/2 1/2 0/2 0/2
TL-1487	Carbamic acid, N-methyl-N-methoxy-2-methyl-5-dimethylaminophenyl ester methiodide	CH_3 $\text{OCONCH}_3\text{OCH}_3$ 	Sc.W.	Mice	2.5 1.0 0.5 0.25	5/7 0/7 1/10 0/10
TL-1300	Carbamic acid, N,N-pentamethylene-2-methyl-5-dimethylaminophenyl ester methiodide	$\text{OCONC}_5\text{H}_{10}$ 	Sc.W.	Mice	80 40 20	1/2 0/2 0/2
TL-1355	Carbamic acid, N,N-pentamethylene-4-methyl-3-dimethylaminophenyl ester methiodide	$\text{OCONC}_5\text{H}_{10}$ 	Sc.W.	Mice	20 10 5 1	2/2 2/2 0/2 0/2

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TABLE 2, Section VIII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect	
TL-1239	Carbamthiotic acid, N-methyl-3-dimethylamino-4-methylphenyl ester methiodide	SCONHCH ₃	Sc.W.	Mice	80	0/2	
					40	0/2	
					20	0/2	
Code	Route and solvent	Species	Effects (at various doses)				
TL-1185	Sc.W.		0.1	0.2	0.3	0.4	1.0
		Rat	0/2	1/2	2/2	...	...
		Rabbit	0/2	1/2	1/2	2/2	...
		G. pig	0/2	2/2	...	...	...
		Dog	0/2	1/2	1/2	...	...
		Cat	0/2	2/2	2/2	...	...
		Sheep	0/1	0/2	0/2	0/2	1/1
		Goat	...	0/2	2/5	0/2	0/2
		Monkey	...	0/2	...	...	...
		TL-1186	Sc.W.		0.05	0.1	0.2
Rat	...			0/2	2/2	...	...
Rabbit	...			0/2	2/2	...	...
G. pig	0/2			2/2	2/2	...	...
Dog	...			0/2	1/2	0/2	...
TL-1188	Sc.W.				0.1	0.2	0.3
		Rat	0/2	1/2	0/2	0/2	...
		Rabbit	0/2	1/2	1/2	...	...
		G. pig	0/2	2/2	...	...	...
		Dog	0/2	2/2	1/2	...	...

IX. Benzene compounds with one carbamate group and one quaternary ammonium group in the para position (including thiocarbamates).

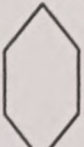
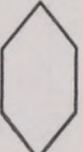
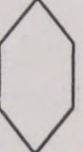
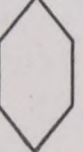
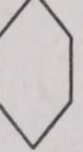
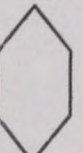
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-943	Carbamic acid, N-methyl-4-aminophenyl ester hydrochloride	OCONHCH_3 	Sc.W.	Mice	80 40 20	2/2 0/2 0/2
		$\text{NH}_2 \cdot \text{HCl}$				
T-1088	Carbamic acid, N-methyl-4-dimethylaminophenyl ester	OCONHCH_3	Sc.	Mice	50	LD_{50}
AR-17	methiodide		Iv.	Mice	2	LD_{80}
TL-1097		$\text{N(CH}_3)_3\text{I}$	Sc.W.	Mice	80 40 20 10 5	2/2 2/2 2/2 0/2 0/2
TL-1469	Carbamic acid, N-methyl-4-dimethylaminophenyl ester ethiodide	OCONHCH_3 	Sc.W.	Mice	40 20 10 5	2/2 2/2 0/2 0/2
		$\text{N(CH}_3)_2\text{C}_2\text{H}_5\text{I}$				

TABLE 2, Section IX (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1456	Carbamic acid, N-methyl-4-dimethylaminophenyl ester allyliodide	OCONHCH_3  $(\text{CH}_3)_2\text{NCH}_2\text{CH}=\text{CH}_2\text{I}$	Sc.W.	Mice	10 5 2.5	2/2 2/2 0/2
TL-1431	Carbamic acid, N-methyl-4-diethylaminophenyl ester methiodide	OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$	Sc.W.	Mice	40 20 10 5	2/2 1/2 0/2 0/2
TL-1432	Carbamic acid, N-methyl-4-diethylaminophenyl ester allyliodide	$\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$ OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_2\text{I}$	Sc.W.	Mice	40 20 10 5	2/2 1/2 0/2 0/2
TL-1430	Carbamic acid, N-methyl-4-diethylaminophenyl ester ethiodide	$\text{CH}_2\text{CH}=\text{CH}_2$ OCONHCH_3  $\text{N}(\text{C}_2\text{H}_5)_3\text{I}$	Sc.W.	Mice	80 40 20 10	2/2 1/2 0/2 0/2
TL-1457	Carbamic acid, N,N-dimethyl-4-dimethylaminophenyl ester ethiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2\text{C}_2\text{H}_5\text{I}$	Sc.W.	Mice	80 40 20	1/2 0/2 0/2
TL-1486	Carbamic acid, N,N-dimethyl-4-dimethylaminophenyl ester β-hydroxyethobromide	$\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2\text{Br}$	Sc.W.	Mice	80 40 20 10	2/2 1/2 0/2 0/2
TL-1470	Carbamic acid, N,N-dimethyl-4-dimethylaminophenyl ester allyliodide	$\text{CH}_2\text{CH}_2\text{OH}$ $\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{CH}_3)_2\text{I}$	Sc.W.	Mice	80 40 20 10	2/2 1/2 0/2 0/2
TL-1458	Carbamic acid, N,N-dimethyl-4-diethylaminophenyl ester methiodide	$\text{CH}_2\text{CH}=\text{CH}_2$ $\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1472	Carbamic acid, N,N-dimethyl-4-diethylaminophenyl ester allyliodide	$\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$ $\text{OCON}(\text{CH}_3)_2$  $\text{N}(\text{C}_2\text{H}_5)_2\text{I}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
		$\text{CH}_2\text{CH}=\text{CH}_2$				

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TABLE 2, Section IX (Continued)

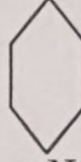
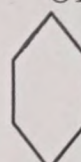
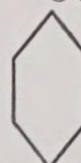
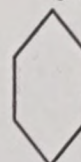
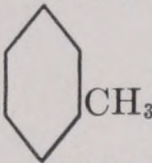
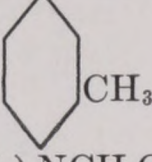
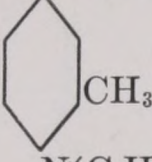
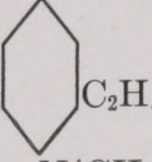
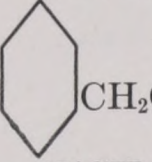
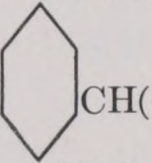
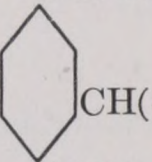
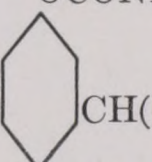
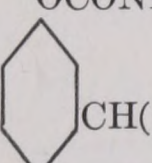
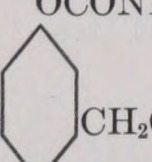
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1471	Carbamic acid, N,N-dimethyl-4-diethylaminophenyl ester ethiodide	OCN(CH ₃) ₂	Sc.W.	Mice	80	0/2
					40	0/2
		N(C ₂ H ₅) ₃ I			20	0/2
TL-1229	Carbamthiolic acid, N-methyl-4-nitrophenyl ester	SCONHCH ₃	Sc.P.	Mice	80	1/2
					40	1/2
		NO ₂			20	0/2
					10	0/2
TL-1258	Carbamthiolic acid, N-methyl-4-dimethylaminophenyl ester methiodide	SCONHCH ₃	Sc.W.	Mice	80	0/2
					40	0/2
		N(CH ₃) ₃ I			20	0/2
TL-1054	Carbamthiolthionic acid, N,N-dimethyl-4-nitrophenyl ester	SCSN(CH ₃) ₂	Sc.P.	Mice	40	0/2
					20	0/2
		NO ₂				
TL-1128	Carbamthiolthionic acid, N,N-dimethyl-4-aminophenyl ester hydrochloride	SCSN(CH ₃) ₂	Sc.W.	Mice	80	0/2
					40	0/2
		NH ₂ ·HCl			20	0/2
TL-1179	Carbamthiolthionic acid, N,N-dimethyl-4-dimethylaminophenyl ester methiodide	SCSN(CH ₃) ₂	Sc.W. (suspension)	Mice	80	1/2
					40	0/2
		N(CH ₃) ₃ I			20	0/2
X. Benzene compounds with one carbamate group and one quaternary ammonium group in the para position and other substituents.						
TL-1478	Phenol, 3-isopropyl-4-dimethylamino-, methiodide	OH	Sc.W.	Mice	80	2/2
					40	2/2
		CH(CH ₃) ₂			20	0/2
		N(CH ₃) ₃ I			10	0/2
TL-1322	Carbamic acid, N-methyl-2-isopropyl-4-dimethylaminophenyl ester methiodide	OCONHCH ₃	Sc.W.	Mice	0.51	LD ₅₀
						
		CH(CH ₃) ₂				
		N(CH ₃) ₃ I				
TL-1446	Carbamic acid, N-methyl-3-methyl-4-dimethylaminophenyl ester methiodide	OCONHCH ₃	Sc.W.	Mice	10	2/2
					5	2/2
		CH ₃			1	1/2
					0.5	1/2
					0.25	0/5
		N(CH ₃) ₃ I				

TABLE 2, Section X (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1447	Carbamic acid, N-methyl-3-methyl-4-dimethylamino-phenyl ester ethiodide	OCONHCH_3 	Sc.W.	Mice	10 5 1 0.5 0.25	2/2 2/2 2/2 2/2 0/5
TL-1448	Carbamic acid, N-methyl-3-methyl-4-dimethylamino-phenyl ester allyliodide	$\text{N(CH}_3)_2\text{C}_2\text{H}_5\text{I}$ OCONHCH_3 	Sc.W.	Mice	0.24 (73 F)	LD_{50}
TL-1454	Carbamic acid, N-methyl-3-methyl-4-diethylamino-phenyl ester methiodide	$(\text{CH}_3)_2\text{NCH}_2\text{CH}=\text{CH}_2\text{I}$ OCONHCH_3 	Sc.W.	Mice	10 5 1 0.5 0.25	2/2 2/2 1/2 0/2 0/2
TL-1467	Carbamic acid, N-methyl-3-ethyl-4-dimethylamino-phenyl ester methiodide	$\text{N(C}_2\text{H}_5)_2\text{CH}_3\text{I}$ OCONHCH_3 	Sc.W.	Mice	0.145 (74 F)	LD_{50}
TL-1468	Carbamic acid, N-methyl-3-propyl-4-dimethylamino-phenyl ester methiodide	$\text{N(CH}_3)_3\text{I}$ OCONHCH_3 	Sc.W.	Mice	0.39 (78 F)	LD_{50}
TL-1381	Carbamic acid, N-methyl-3-isopropyl-4-dimethylamino-phenyl ester hydrochloride	$\text{N(CH}_3)_3\text{I}$ OCONHCH_3 	Sc.W.	Mice	10 5 2.5 1.0 0.5	2/2 2/2 2/2 1/2 0/2
TL-1327	Carbamic acid, N-methyl-3-isopropyl-4-dimethylamino-phenyl ester methiodide	$\text{N(CH}_3)_2\cdot\text{HCl}$ OCONHCH_3 	Sc.W.	Mice	0.067 0.070 (79 F)	LD_{50} LD_{50}
	Prep. 2	$\text{N(CH}_3)_3\text{I}$	pH 4	Mice	0.064	LD_{50}
TL-1345	Carbamic acid, N-methyl-3-isopropyl-4-dimethylamino-phenyl ester methochloride	OCONHCH_3 	Sc.W.	Mice	0.045*	LD_{50}
	Prep. 2		Sc.W.	Mice	0.047†	LD_{50}
	Prep. 2		Sc.W. pH 4	Mice	0.050‡	LD_{50}
	Prep. 2		Sc.W.	Rats	0.103§	LD_{50}
		$\text{N(CH}_3)_3\text{Cl}$	(See p. 234)			
TL-1522	Carbamic acid, N-methyl-3-isopropyl-4-dimethylamino-phenyl ester allyl bromide	OCONHCH_3 	Sc.W.	Mice	0.057†	LD_{50}
TL-1475	Carbamic acid, N-methyl-3-butyl-4-dimethylamino-phenyl ester methiodide	$(\text{CH}_3)_2\text{NCH}_2\text{CH}=\text{CH}_2\text{Br}$ OCONHCH_3 	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
		$\text{N(CH}_3)_3\text{I}$				

* At 90 F.

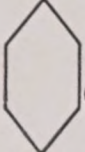
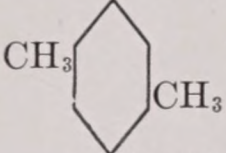
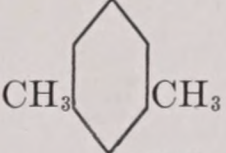
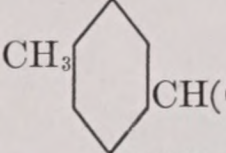
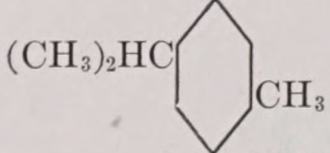
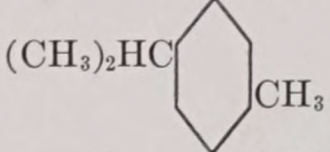
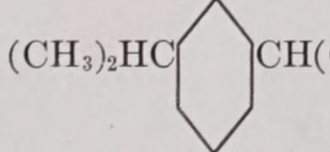
† At 87 F.

‡ At 78 F.

§ At 81 F.

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TABLE 2, Section X (Continued)

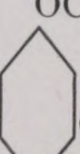
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1476	Carbamic acid, N-methyl-3-amyl-4-dimethylamino-phenyl ester methiodide	OCONHCH_3  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ $\text{N(CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-1416	Carbamic acid, N-methyl-3-cyclopentyl-4-dimethylaminophenyl ester methiodide	OCONHCH_3  CH_2-CH_2 CH_2-CH_2 $\text{N(CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-1490	Carbamic acid, N-methyl-3-hexyl-4-dimethylamino-phenyl ester methiodide	OCONHCH_3  C_6H_{13} $\text{N(CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 2.5 1.0 0.5	2/2 2/2 2/2 0/2 0/5
TL-1489	Carbamic acid, N-methyl-2,5-dimethyl-4-dimethylamino-phenyl ester ethiodide	OCONHCH_3  $\text{N(CH}_3)_2\text{C}_2\text{H}_5\text{I}$	Sc.W.	Mice	0.325*	LD_{50}
TL-1254	Carbamic acid, N-methyl-3,5-dimethyl-4-dimethylamino-phenyl ester hydroiodide	OCONHCH_3  $\text{N(CH}_3)_2\text{HI}$	Sc.W.	Mice	80 40 20 10	2/2 2/2 0/2 0/2
TL-1482	Carbamic acid, N-methyl-4-dimethylaminocarvacryl ester ethiodide	OCONHCH_3  $\text{N(CH}_3)_2\text{C}_2\text{H}_5\text{I}$	Sc.W.	Mice	0.145†	LD_{50}
SB-26	Carbamic acid, N-methyl-4-dimethylaminothymyl ester hydrochloride	OCONHCH_3  $\text{N(CH}_3)_2 \cdot \text{HCl}$	Sc.	Mice	23	LD_{50}
SB-27	Carbamic acid, N-methyl-4-dimethylaminothymyl ester methiodide	OCONHCH_3  $\text{N(CH}_3)_3\text{I}$	Sc.	Mice	0.22	LD_{50}
TL-1451	Carbamic acid, N-methyl-2,6-diisopropyl-4-dimethylaminophenyl ester methiodide	OCONHCH_3  $\text{N(CH}_3)_3\text{I}$	Sc.W.	Mice	80 40 20	2/2 0/2 0/2
SB-2	Carbamic acid, N,N-dimethyl-4-dimethylamino-2-methylphenyl ester hydrochloride	$\text{OCON(CH}_3)_2$  $\text{N(CH}_3)_2 \cdot \text{HCl}$	Sc.	Mice	>400	LD_{50}

* At 75 F.

† At 73 F.

SECRET

TABLE 2, Section X (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
SB-3	Carbamic acid, N,N-dimethyl-4-dimethylamino-2-methylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  CH_3 $\text{N}(\text{CH}_3)_3\text{I}$	Sc.	Mice	6.5	LD_{50}
TL-1313	Carbamic acid, N,N-dimethyl-4-dimethylamino-2-isopropylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}(\text{CH}_3)_2$ $\text{N}(\text{CH}_3)_3\text{I}$	Sc.W.	Mice	0.47	LD_{50}
SB-4	Carbamic acid, N,N-dimethyl-4-dimethylamino-3-methylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  CH_3 $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	Sc.	Mice	105	LD_{50}
SB-5	Carbamic acid, N,N-dimethyl-4-dimethylamino-3-methylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  CH_3 $\text{N}(\text{CH}_3)_3\text{I}$	Sc.	Mice	13.0	LD_{50}
TL-1449	Carbamic acid, N,N-dimethyl-3-methyl-4-dimethylamino-phenyl ester ethiodide	$\text{OCON}(\text{CH}_3)_2$  CH_3 $\text{N}(\text{CH}_3)_2\text{C}_2\text{H}_5\text{I}$	Sc.W.	Mice	10 5 1	$1/2$ $0/2$ $0/2$
TL-1455	Carbamic acid, N,N-dimethyl-3-methyl-4-diethylamino-phenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  CH_3 $\text{N}(\text{C}_2\text{H}_5)_2\text{CH}_3\text{I}$	Sc.W.	Mice	80 40 20 10	$1/2$ $1/2$ $0/2$ $0/2$
SB-6	Carbamic acid, N,N-dimethyl-4-dimethylamino-3-ethylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  C_2H_5 $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	Sc.	Mice	45	LD_{50}
SB-7 TL-1412	Carbamic acid, N,N-dimethyl-4-dimethylamino-3-ethylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  C_2H_5 $\text{N}(\text{CH}_3)_3\text{I}$	Sc. Sc.W.	Mice Mice	1.15 10 5 1 0.5	LD_{50} $2/2$ $2/2$ $1/2$ $0/2$
SB-8 TL-599	Carbamic acid, N,N-dimethyl-4-dimethylamino-5-isopropylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}(\text{CH}_3)_2$ $\text{N}(\text{CH}_3)_3\text{I}$	Sc. Sc.W. Sc.W. Ip.W. Ip.W. Ip.W.	Mice Mice Mice Mice Mice Mice	0.075 0.080 0.089 0.168 0.220 0.265	LD_{50} LD_{50} LD_{50} LD_{50} LD_{50} LD_{50}
TL-1460	Carbamic acid, N,N-dimethyl-3-propyl-4-dimethylamino-phenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{CH}_2\text{CH}_3$ $\text{N}(\text{CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 1 0.50 0.25	$2/2$ $2/2$ $2/2$ $0/2$ $0/2$

(See p. 234)

TABLE 2, Section X (Continued)

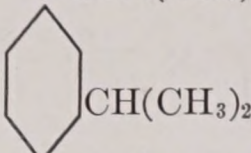
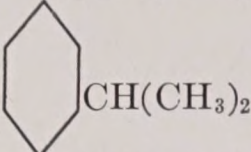
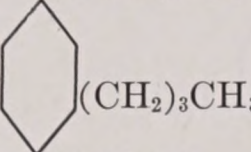
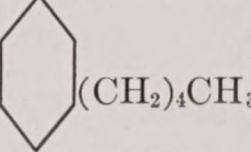
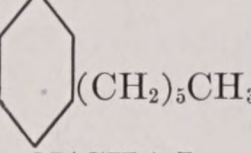
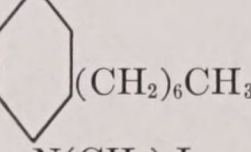
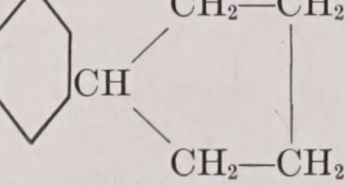
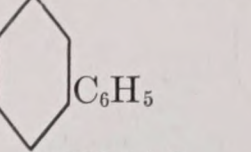
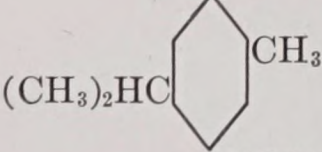
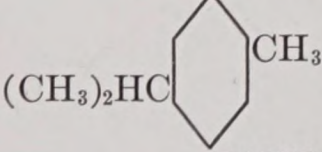
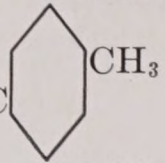
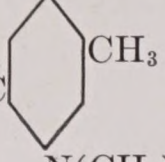
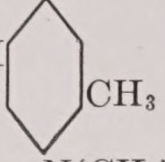
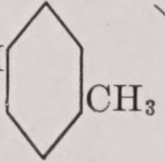
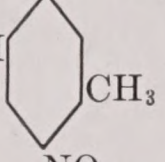
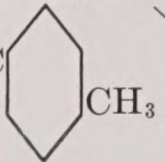
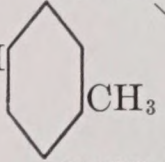
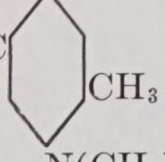
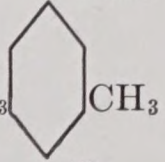
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1443	Carbamic acid, N,N-dimethyl-3-isopropyl-4-dimethylaminophenyl ester methochloride	$\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	0.065 (73 F)	LD_{50}
TL-1521	Carbamic acid, N,N-dimethyl-3-isopropyl-4-dimethylaminophenyl ester ethiodide	$\text{N}(\text{CH}_3)_3\text{Cl}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	0.182 (71 F)	LD_{50}
TL-1461	Carbamic acid, N,N-dimethyl-3-butyl-4-dimethylaminophenyl ester methiodide	$\text{N}(\text{CH}_3)_2\text{C}_2\text{H}_5\text{I}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-1462	Carbamic acid, N,N-dimethyl-3-amyl-4-dimethylaminophenyl ester methiodide	$\text{N}(\text{CH}_3)_3\text{I}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-1463	Carbamic acid, N,N-dimethyl-3-hexyl-4-dimethylaminophenyl ester methiodide	$\text{N}(\text{CH}_3)_3\text{I}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 0/2 0/2
TL-1464	Carbamic acid, N,N-dimethyl-3-heptyl-4-dimethylaminophenyl ester methiodide	$\text{N}(\text{CH}_3)_3\text{I}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	80 40 20 10	2/2 2/2 0/2 0/2
TL-1417	Carbamic acid, N,N-dimethyl-3-cyclopentyl-4-dimethylaminophenyl ester methiodide	$\text{N}(\text{CH}_3)_3\text{I}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 2/2 0/2
TL-1466	Carbamic acid, N,N-dimethyl-3-phenyl-4-dimethylaminophenyl ester methiodide	$\text{N}(\text{CH}_3)_3\text{I}$ $\text{OCON}(\text{CH}_3)_2$ 	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
SB-28	Carbamic acid, N-methyl-4-dimethylaminocarvacryl ester hydrochloride	$\text{N}(\text{CH}_3)_3\text{I}$ OCONHCH_3 	Sc.	Mice	2.1	LD_{50}
SB-29	Carbamic acid, N-methyl-4-dimethylaminocarvacryl ester methiodide	$\text{N}(\text{CH}_3)_2\cdot\text{HCl}$ OCONHCH_3 	Sc.	Mice	0.09	LD_{50}

TABLE 2, Section X (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
SB-11	Carbamic acid, N,N-dimethyl-4-dimethylaminocarvacryl ester hydrochloride	$\begin{array}{c} \text{OCON}(\text{CH}_3)_2 \\ \\ (\text{CH}_3)_2\text{HC} \text{---} \text{C}_6\text{H}_3 \text{---} \text{CH}_3 \end{array}$	Sc.	Mice	20	LD_{50}
SB-12	Carbamic acid, N,N-dimethyl-4-dimethylaminocarvacryl ester methiodide	$\begin{array}{c} \text{OCON}(\text{CH}_3)_2 \\ \\ (\text{CH}_3)_2\text{HC} \text{---} \text{C}_6\text{H}_3 \text{---} \text{CH}_3 \\ \\ \text{N}(\text{CH}_3)_2 \cdot \text{HCl} \end{array}$	Sc.	Mice	0.24	LD_{50}
SB-9	Carbamic acid, N,N-dimethyl-4-dimethylaminothymyl ester hydrochloride	$\begin{array}{c} \text{OCON}(\text{CH}_3)_2 \\ \\ (\text{CH}_3)_2\text{HC} \text{---} \text{C}_6\text{H}_3 \text{---} \text{CH}_3 \\ \\ \text{N}(\text{CH}_3)_3\text{I} \end{array}$	Sc.	Mice	160	LD_{50}
SB-10	Carbamic acid, N,N-dimethyl-4-dimethylaminothymyl ester methiodide	$\begin{array}{c} \text{OCON}(\text{CH}_3)_2 \\ \\ (\text{CH}_3)_2\text{HC} \text{---} \text{C}_6\text{H}_3 \text{---} \text{CH}_3 \\ \\ \text{N}(\text{CH}_3)_2 \cdot \text{HCl} \end{array}$	Sc.	Mice	0.72	LD_{50}
TL-1195	Carbamic acid, N,N-dimethyl-3,5-dimethyl-4-nitrophenyl ester	$\begin{array}{c} \text{OCON}(\text{CH}_3)_2 \\ \\ \text{CH}_3 \text{---} \text{C}_6\text{H}_2(\text{CH}_3)_2 \text{---} \text{CH}_3 \\ \\ \text{NO}_2 \end{array}$	Sc.P.	Mice	80 40 20	0/2 0/2 0/2
TL-1253	Carbamic acid, N,N-dimethyl-3,5-dimethyl-4-dimethylaminophenyl ester hydroiodide	$\begin{array}{c} \text{OCON}(\text{CH}_3)_2 \\ \\ \text{CH}_3 \text{---} \text{C}_6\text{H}_2(\text{CH}_3)_2 \text{---} \text{CH}_3 \\ \\ \text{N}(\text{CH}_3)_2\text{HI} \end{array}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1377	Carbamic acid, N-ethyl-3,5-diisopropyl-4-dimethylaminophenyl ester	$\begin{array}{c} \text{OCONHC}_2\text{H}_5 \\ \\ (\text{CH}_3)_2\text{CH} \text{---} \text{C}_6\text{H}_2(\text{CH}_3)_2 \text{---} \text{CH}(\text{CH}_3)_2 \\ \\ \text{N}(\text{CH}_3)_2 \end{array}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1077	Carbamic acid, N,N-diethyl-3,5-dimethyl-4-nitrosophenyl ester	$\begin{array}{c} \text{OCON}(\text{C}_2\text{H}_5)_2 \\ \\ \text{CH}_3 \text{---} \text{C}_6\text{H}_2(\text{CH}_3)_2 \text{---} \text{CH}_3 \\ \\ \text{NO} \end{array}$	Sc.O.	Mice	80 40 20	0/2 0/2 0/2
TL-1197	Carbamic acid, N,N-diethyl-3,5-dimethyl-4-nitrophenyl ester	$\begin{array}{c} \text{OCON}(\text{C}_2\text{H}_5)_2 \\ \\ \text{CH}_3 \text{---} \text{C}_6\text{H}_2(\text{CH}_3)_2 \text{---} \text{CH}_3 \\ \\ \text{NO}_2 \end{array}$	Sc.P.	Mice	80 40 20	0/2 0/2 0/2
TL-967	Carbamic acid, N,N-diethyl-4-dimethylaminothymyl ester methiodide	$\begin{array}{c} \text{OCON}(\text{C}_2\text{H}_5)_2 \\ \\ (\text{CH}_3)_2\text{HC} \text{---} \text{C}_6\text{H}_3 \text{---} \text{CH}_3 \\ \\ \text{N}(\text{CH}_3)_3\text{I} \end{array}$	Sc.W.	Mice	80 40 20 10	2/2 1/2 0/2 0/2

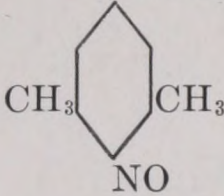
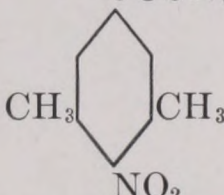
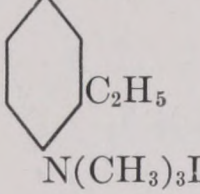
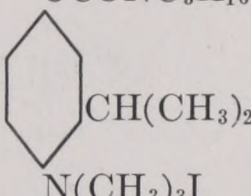
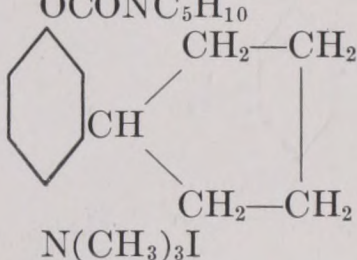
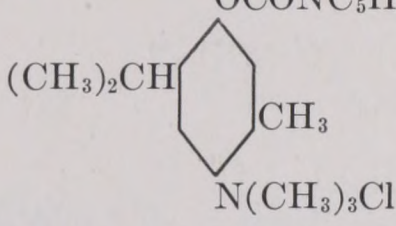
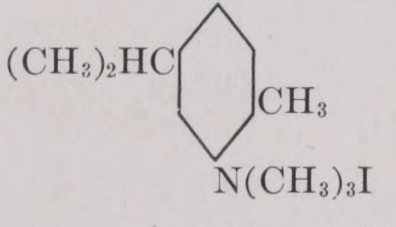
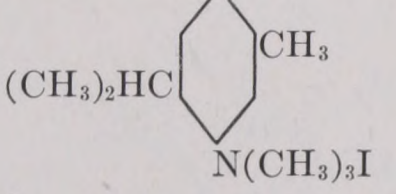
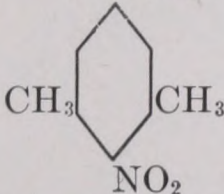
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TABLE 2, Section X (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-778	Carbamic acid, N,N-diethylenoxy-4-dimethylamino-carvacryl ester methochloride	OCONC ₄ H ₉ O	Sc.W.	Mice	80	0/2
		(CH ₃) ₂ HC  CH ₃			40	0/2
		N(CH ₃) ₃ Cl			20	0/2
TL-776	Carbamic acid, N,N-diethylenoxy-4-dimethylamino-carvacryl ester methiodide	OCONC ₄ H ₉ O	Sc.W.	Mice	80	1/2
		(CH ₃) ₂ HC  CH ₃			40	0/2
		N(CH ₃) ₃ I			10	0/2
TL-1073	Carbamic acid, N,N-bis(2-chloroethyl)-4-dimethylaminothymyl ester methochloride	OCON(CH ₂ CH ₂ Cl) ₂	Sc.W.	Mice	80	1/2
		(CH ₃) ₂ CH  CH ₃			40	1/2
		N(CH ₃) ₃ Cl			20	0/2
					10	0/2
TL-1079	Carbamic acid, N-(2-chloroethyl)-N-ethyl-4-nitrosothymyl ester	OCON  CH ₃	Sc.O.	Mice	80	0/2
		C ₂ H ₅			40	0/2
		CH ₂ CH ₂ Cl			20	0/2
		NO				
TL-1080	Carbamic acid, N,N-bis(2-chloroethyl)-4-nitrothymyl ester	OCON(CH ₂ CH ₂ Cl) ₂	Sc.O.	Mice	80	0/2
		(CH ₃) ₂ CH  CH ₃			40	0/2
		NO ₂			20	0/2
TL-969	Carbamic acid, N-(2-chloroethyl)-N-ethyl-4-dimethylaminothymyl ester methiodide	OCON  CH ₃	Sc.W.	Mice	80	2/2
		C ₂ H ₅			40	0/2
		CH ₂ CH ₂ Cl			20	0/2
		N(CH ₃) ₃ I				
TL-1074	Carbamic acid, N-(2-chloroethyl)-N-ethyl-4-dimethylaminothymyl ester methochloride	OCON  CH ₃	Sc.W.	Mice	80	2/2
		C ₂ H ₅			40	2/2
		CH ₂ CH ₂ Cl			20	0/2
		N(CH ₃) ₃ Cl			10	0/2
TL-1048	Carbamic acid, N,N-bis(2-chloroethyl)-4-dimethylaminothymyl ester methiodide	OCON(C ₂ H ₄ Cl) ₂	Sc.W.	Mice	80	0/2
		(CH ₃) ₂ HC  CH ₃			40	0/2
		N(CH ₃) ₃ I			20	0/2
TL-1198	Carbamic acid, N-(2-chloroethyl)-N-ethyl-3,5-dimethyl-4-nitrophenyl ester	OCON  CH ₃	Sc.P.	Mice	80	0/2
		C ₂ H ₅			40	0/2
		CH ₂ CH ₂ Cl			20	0/2
		NO ₂				

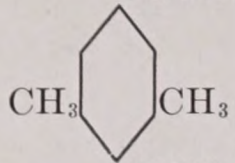
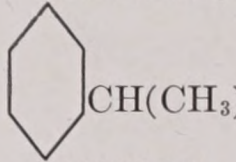
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TABLE 2, Section X (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1075	Carbamic acid, N,N-bis(2-chloroethyl)-3,5-dimethyl-4-nitrosophenyl ester		Sc.O.	Mice	80	1/2
					40	0/2
					20	0/2
						0/2
TL-1255	Carbamic acid, N,N-bis(2-chloroethyl)-3,5-dimethyl-4-nitrophenyl ester trihydrate		Sc.P.	Mice	80	0/2
					40	0/2
					20	0/2
						0/2
TL-1413	Carbamic acid, N,N-pentamethylene-3-ethyl-4-dimethylaminophenyl ester methiodide		Sc.W.	Mice	80	2/2
					40	2/2
					20	2/2
					10	0/2
					5	0/2
TL-1414	Carbamic acid, N,N-pentamethylene-3-isopropyl-4-dimethylaminophenyl ester methiodide		Sc.W.	Mice	0.51	LD ₅₀
					(78 F)	
TL-1418	Carbamic acid, N,N-pentamethylene-3-cyclopentyl-4-dimethylaminophenyl ester methiodide		Sc.W.	Mice	80	2/2
					40	2/2
					20	1/2
					10	0/2
						0/2
TL-1049	Carbamic acid, N,N-pentamethylene-4-dimethylaminothymyl ester methochloride		Sc.P.	Mice	0.36	LD ₅₀
TL-968	Carbamic acid, N,N-pentamethylene-4-dimethylaminothymyl ester methiodide		Sc.W.	Mice	0.44	LD ₅₀
TL-777	Carbamic acid, N,N-pentamethylene-4-dimethylaminocarvacryl ester methiodide		Sc.W.	Mice	3	2/3
					2	0/3
			Iv.W.	Mice	1	0/3
					3	3/5
					2	0/5
TL-1196	Carbamic acid, N,N-pentamethylene-3,5-dimethyl-4-nitrophenyl ester		Sc.P.	Mice	80	1/2
					40	1/2
					20	0/2
					10	0/2
						0/2

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TABLE 2, Section X (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1260	Carbamic acid, N,N-pentamethylene-3,5-dimethyl-4-dimethylaminophenyl ester hydroiodide	$\text{OCONC}_5\text{H}_{10}$  $\text{N}(\text{CH}_3)_2 \text{HI}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1465	Carbamic acid, N-phenyl-3-isopropyl-4-dimethylaminophenyl ester methiodide	$\text{OCONHC}_6\text{H}_5$  $\text{N}(\text{CH}_3)_3 \text{I}$	Sc.W.	Mice	40 20 10 5 2.5	2/2 2/2 2/2 1/2 0/2

Code	Route and solvent	Species	Effects (at various doses)				
TL-1448	Sc.W.		0.2	0.3	0.5	1.0	
		Rat	...	0/2	2/2	...	...
		Rabbit	...	0/2	2/2	...	...
		G. pig	0/2	1/2	1/2	2/2	...
TL-1345 (1st sample)	Sc.W.		0.025	0.05	0.1	0.15	0.2
		G. pig	0/2	1/2	2/2	...	...
		Rabbit	0/2	2/2	1/2	4/4	...
		Dog	0/2	1/2	1/2	2/2	...
		Cat	...	0/2	1/2	2/2	2/2
		Monkey	...	...	0/2	...	3/3
SB-8	Sc.W.		0.1	0.2	0.3		
TL-599		Rat	...	3/6	6/6	...	...
		Rabbit	...	1/3	2/3	...	...
		G. pig	1/4	4/5	5/5	...	...
		Dog	0/2	2/3	3/5	...	...
		Cat	...	0/3	2/2	...	...

XI. Benzene compounds with one carbamate group and an alkyl side chain having a quaternary ammonium group.

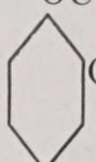
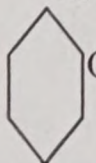
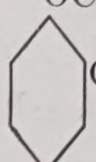
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1180	Carbamic acid, N-methyl-2-dimethylaminomethylphenyl ester methiodide	OCONHCH_3  $\text{CH}_2\text{N}(\text{CH}_3)_3 \text{I}$	Sc. Sc.	Mice Rabbit	7.2 3.5	LD_{50}
AR-39	Carbamic acid, N,N-dimethyl-2-diethylaminomethylphenyl ester hydrochloride	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2 \cdot \text{HCl}$	Iv.	Mice	1.5	LD_{80}
AR-40	Carbamic acid, N,N-dimethyl-2-diethylaminomethylphenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2 \text{I}$	Iv.	Mice	0.5	LD_{80}

TABLE 2, Section XI (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-(?)	Carbamic acid, N-methyl-N-(N'-methylcarbamy)-2-dimethylaminomethylphenyl ester methiodide		Sc.	Mice	343	LD_{50}
T-2065	Carbamic acid, N-methyl-2-(1-dimethylamino- <i>n</i> -propyl)phenyl ester methiodide		Sc.	Mice	10	LD_{50}
T-2068	Carbamic acid, N-methyl-2-(1-dimethylamino- <i>n</i> -propyl)phenyl ester hydrochloride		Sc.	Mice	40	LD_{50}
T-1890	Carbamic acid, N-methyl-2-dimethylaminomethyl-6-methylphenyl ester hydrochloride		?	Mice	350	LD_{50}
T-1891	Carbamic acid, N-methyl-2-dimethylaminomethyl-5-methylphenyl ester hydrochloride		?	Mice	150	LD_{50}
T-1892	Carbamic acid, N-methyl-2-dimethylaminomethyl-4-methylphenyl ester hydrochloride		?	Mice	140	LD_{50}
T-1893	Carbamic acid, N-methyl-2-dimethylaminomethyl-4-methylphenyl ester methiodide		?	Mice	75	LD_{50}
T-1847	Carbamic acid, N-methyl-2-(α-dimethylaminoethyl)-4-methylphenyl ester hydrochloride		?	Mice	70	LD_{50}
T-1846	Carbamic acid, N-methyl-2-(α-dimethylaminoethyl)-4-methylphenyl ester methiodide		?	Mice	12	LD_{50}
T-1824	Carbamic acid, N-methyl-3-(dimethylaminomethyl)-phenyl ester hydrochloride		?	Mice	10	LD_{50}

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TABLE 2, Section XI (Continued)

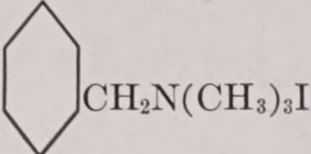
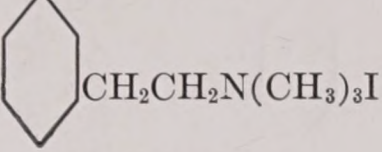
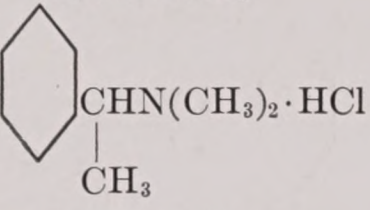
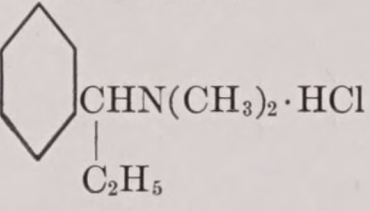
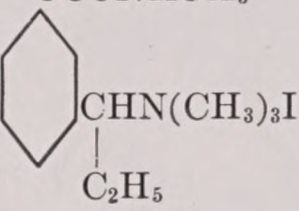
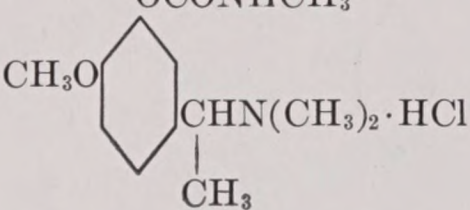
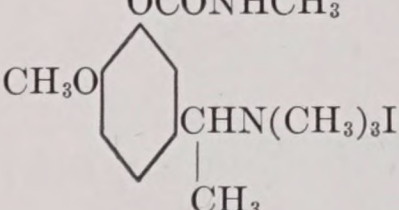
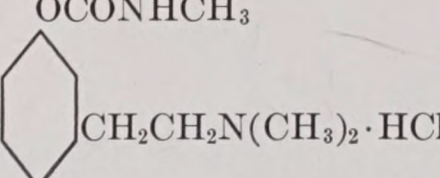
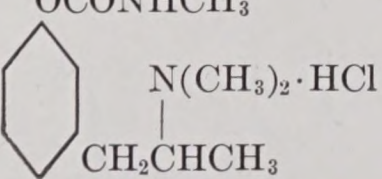
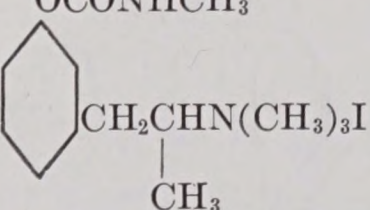
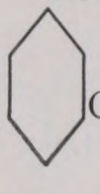
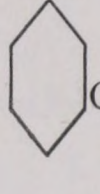
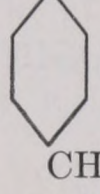
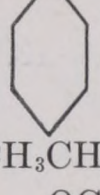
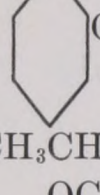
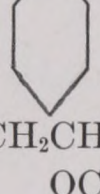
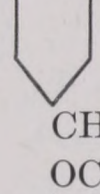
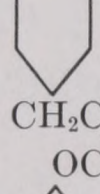
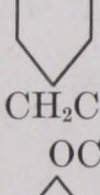
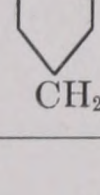
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1825	Carbamic acid, N-methyl-3-(dimethylaminomethyl)-phenyl ester methiodide	OCONHCH_3 	?	Mice	7	LD_{50}
T-1887 ? T-1939 ?	Carbamic acid, N-methyl-3-(β -dimethylaminoethyl)-phenyl ester methiodide	OCONHCH_3 	? ?	Mice Mice ca.	100 7.5-10	LD_{50} LD_{50}
AR-28 T-1843	Carbamic acid, N-methyl-3-(α -dimethylaminoethyl)-phenyl ester hydrochloride (miotine)	OCONHCH_3 	Iv. Iv. Sc.	Mice Mice Rabbit G. pig Rat Mice	1.0 0.5 1.0 ± 0.5 1.0 ± 0.5 1.0 ± 0.5 1.0 ± 0.5	LD_{80} LD_{50} LD_{50}
T-1894	Carbamic acid, N-methyl-3-(α -dimethylaminopropyl)-phenyl ester hydrochloride	OCONHCH_3 	?	Mice	3.0	LD_{50}
T-1895	Carbamic acid, N-methyl-3-(α -dimethylaminopropyl)-phenyl ester methiodide	OCONHCH_3 	?	Mice	5.0	LD_{50}
AR-29	Carbamic acid, N-methyl-3-(α -dimethylaminoethyl)-6-methoxyphenyl ester hydrochloride	OCONHCH_3 	Iv.	Mice	6	LD_{80}
AR-30	Carbamic acid, N-methyl-3-(α -dimethylaminoethyl)-6-methoxyphenyl ester methiodide	OCONHCH_3 	Iv.	Mice	5	LD_{80}
T-1886 ? T-1938 ?	Carbamic acid, N-methyl-3-(β -dimethylaminoethyl)-phenyl ester hydrochloride	OCONHCH_3 	? ?	Mice Mice	35 Approx. 3.0	LD_{50} LD_{50}
T-2040	Carbamic acid, N-methyl-3-(2-dimethylamino- <i>n</i> -propyl)-phenyl ester hydrochloride	OCONHCH_3 	Sc.	Mice	Approx. 16	LD_{50}
T-2064	Carbamic acid, N-methyl-3-(2-dimethylamino- <i>n</i> -propyl)-phenyl ester methiodide	OCONHCH_3 	Sc.	Mice	0.6	LD_{50}

TABLE 2, Section XI (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-2038	Carbamic acid, N-methyl-3-(3-dimethylamino- <i>n</i> -butyl)-phenyl ester hydrochloride	 OCONHCH_3 $\text{N(CH}_3)_2 \cdot \text{HCl}$ $\text{CH}_2\text{CH}_2\text{CH}$ CH_3	Sc.	Mice	9	LD_{50}
T-2039	Carbamic acid, N-methyl-3-(3-dimethylamino- <i>n</i> -butyl)-phenyl ester methiodide	 OCONHCH_3 $\text{N(CH}_3)_3\text{I}$ $\text{CH}_2\text{CH}_2\text{CH}$ CH_3	Sc.	Mice	10	LD_{50}
T-1845	Carbamic acid, N-methyl-4-dimethylaminomethyl-phenyl ester hydrochloride	 OCONHCH_3 $\text{CH}_2\text{N(CH}_3)_2 \cdot \text{HCl}$	?	Mice	60	LD_{50}
T-1844	Carbamic acid, N-methyl-4-(α -dimethylaminoethyl)-phenyl ester hydrochloride	 OCONHCH_3 $\text{CH}_3\text{CHN(CH}_3)_2 \cdot \text{HCl}$	?	Mice	25	LD_{50}
AR-28a	Carbamic acid, N-methyl-4-(α -dimethylaminoethyl)-2-methoxyphenyl ester hydrochloride	 OCONHCH_3 OCH_3 $\text{CH}_3\text{CHN(CH}_3)_2 \cdot \text{HCl}$	Iv.	Mice	1-1.5	LD_{80}
T-1896	Carbamic acid, N-methyl-4-(α -dimethylaminopropyl)-phenyl ester methiodide	 OCONHCH_3 $\text{CH}_3\text{CH}_2\text{CHN(CH}_3)_2 \cdot \text{HCl}$	?	Mice	300	LD_{50}
T-1834	Carbamic acid, N-methyl-4-(β -dimethylaminoethyl)-phenyl ester hydrochloride	 OCONHCH_3 $\text{CH}_3\text{CH}_2\text{CHN(CH}_3)_2 \cdot \text{HCl}$	?	Mice	10	LD_{50}
AR-41	Carbamic acid, N,N-dimethyl-4-(β -dimethylaminoethyl)-phenyl ester hydrochloride	 $\text{OCON(CH}_3)_2$ $\text{CH}_2\text{CH}_2\text{N(CH}_3)_2 \cdot \text{HCl}$	Iv.	Mice	15	LD_{80}
AR-42	Carbamic acid, N,N-dimethyl-4-(β -dimethylaminoethyl)-phenyl ester methiodide	 $\text{OCON(CH}_3)_2$ $\text{CH}_2\text{CH}_2\text{N(CH}_3)_2 \cdot \text{HCl}$	Iv.	Mice	55	LD_{80}
T-1935	Carbamic acid, N-methyl-4-(γ -dimethylaminopropyl)-phenyl ester hydrochloride	 OCONHCH_3 $\text{CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_3)_2 \cdot \text{HCl}$	?	Mice	5-7.5	LD_{50}

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TABLE 2, Section XI (Continued)

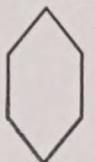
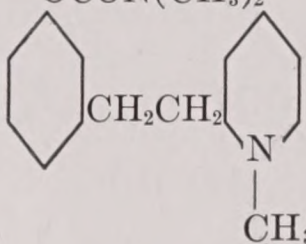
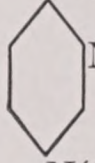
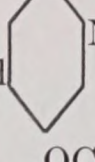
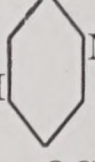
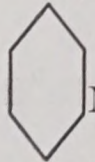
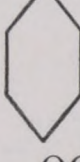
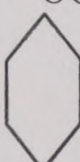
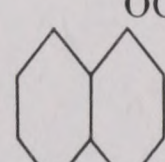
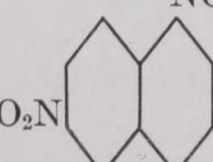
Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1936	Carbamic acid, N-methyl-4-(γ -dimethylaminopropyl)-phenyl ester methiodide	OCONHCH_3  $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{I}$	?	Mice	Approx. 50	LD_{50}
T-1981	Carbamic acid, N-methyl-4-(γ -dimethylamino- <i>n</i> -butyl)-phenyl ester hydrochloride	OCONHCH_3  $\text{CH}_2\text{CH}_2\text{CHN}(\text{CH}_3)_2 \cdot \text{HCl}$	Sc.	Mice	100	LD_{50}
T-1982	Carbamic acid, N-methyl-4-(γ -dimethylamino- <i>n</i> -butyl)-phenyl ester methiodide	OCONHCH_3  $\text{CH}_2\text{CH}_2\text{CHN}(\text{CH}_3)_3\text{I}$	Sc.	Mice	40	LD_{50}
TL-1415	Carbamic acid, N,N-dimethyl-3-(β -2-pyridylethyl)phenyl ester methiodide	$\text{OCON}(\text{CH}_3)_2$  $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{I}$	Sc.W.	Mice	0.33 (78 F)	LD_{50}
XII. Benzene compounds with one carbamate group and two quaternary ammonium groups.						
T-1827	Carbamic acid, N-methyl-2,4- <i>bis</i> (dimethylamino)phenyl ester dihydrochloride	OCONHCH_3  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	?	Mice	60	LD_{50}
T-1826	Carbamic acid, N-methyl-2,4- <i>bis</i> (dimethylamino)phenyl ester dimethiodide	OCONHCH_3  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	?	Mice	7	LD_{50}
T-1809 T-1811	Carbamic acid, N-methyl-2,5- <i>bis</i> (dimethylamino)phenyl ester dihydrochloride	OCONHCH_3  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	?	Mice	50-75	LD_{50}
T-1810	Carbamic acid, N-methyl-2,5- <i>bis</i> (dimethylamino)phenyl ester dimethiodide	OCONHCH_3  $\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$	?	Mice	500-1,000	LD_{50}
AR-27	Carbamic acid, N-methyl-3-[methyl-(β -diethylaminoethyl)-amino]phenyl ester hydrochloride	OCONHCH_3  $\text{NCH}_2\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2 \cdot \text{HCl}$	Iv.	Mice	0.1	LD_{80}

TABLE 2, Section XII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1780	Carbamic acid, N-methyl-4-[methyl-(β -diethylaminoethyl)-amino]phenyl ester dihydrobromide	OCONHCH_3  $\text{CH}_3\text{—N—CH}_2\text{CH}_2\text{N(C}_2\text{H}_5)_2 \cdot \text{HBr}$	?	Mice	16	LD_{50}
T-1779	Carbamic acid, N-methyl-4-[methyl-(β -diethylaminoethyl)-amino]phenyl ester monomethiodide	OCONHCH_3  $\text{CH}_3\text{—N—CH}_2\text{CH}_2\text{N(C}_2\text{H}_5)_2$	?	Mice	100	LD_{50}
T-1833	Carbamic acid, N-methyl-5-dimethylamino-2-dimethylaminomethylphenyl ester dihydrochloride	OCONHCH_3  $(\text{CH}_3)_2\text{N} \cdot \text{HCl}$	?	Mice	500–2,500	LD_{50}
XIII. Benzene compound with one carbamate group and one sulfonium or arsonium group.						
TL-1306	Carbamic acid, N-methyl-3-methylthiophenyl ester methosulfate	OCONHCH_3  $\text{S(CH}_3)_2\text{SO}_4\text{CH}_3$	Sc.W.	Mice	0.370	LD_{50}
TL-1452	Carbamic acid, N-methyl-2-dimethylarsinophenyl ester methiodide	OCONHCH_3  $\text{As(CH}_3)_3\text{I}$	Sc.W.	Mice	80 40 20	1/2 0/2 0/2
TL-1479	Carbamic acid, N,N-dimethyl-3-dimethylarsinophenyl ester methiodide	$\text{OCON(CH}_3)_2$  $\text{As(CH}_3)_3\text{I}$	Sc.W.	Mice	10 5 1 0.5	2/2 2/2 2/2 0/2
TL-1504	Carbamic acid, N-methyl-3-diethylarsinophenyl ester methiodide	OCONHCH_3  $\text{As(C}_2\text{H}_5)_2\text{CH}_3\text{I}$	Sc.W.	Mice	1.0 0.5 0.25 0.125	2/2 2/2 0/2 0/2
TL-1459	Carbamic acid, N,N-dimethyl-4-dimethylarsinophenyl ester methiodide	$\text{OCON(CH}_3)_2$  $\text{As(CH}_3)_3\text{I}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
XIV. Carbamates of naphthalene derivatives.						
TL-1096	Carbamic acid, N-methyl-2,4-dinitro-1-naphthyl ester	OCONHCH_3  NO_2	Sc.P.	Mice	80 40 20	2/2 0/2 0/2
TL-1053	Carbamic acid, N-methyl-1,6-dinitro-2-naphthyl ester	OCONCH_3  O_2N	Sc.P.	Mice	40 20 10	1/2 0/2 0/2

SECRET

TABLE 2, Section XIV (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1889	Carbamic acid, N-methyl-5,6,7,8-tetrahydro-5-dimethylamino-2-naphthyl ester methiodide		?	Mice	20	LD ₅₀
T-1888	Carbamic acid, N-methyl-5,6,7,8-tetrahydro-5-dimethylamino-2-naphthyl ester hydrobromide		?	Mice	4.0	LD ₅₀
TL-1406	Carbamic acid, N-methyl-5,6,7,8-tetrahydro-4-dimethylamino-1-naphthyl ester methiodide		Sc.W.	Mice	0.31 (74 F)	LD ₅₀
XV. Carbamates of quinoline and isoquinoline derivatives.						
T-1934	Carbamic acid, N-methyl-8-quinolinylnyl ester hydrochloride		?	Mice	Approx. 500	LD ₅₀
AR-37	Carbamic acid, N,N-dimethyl-8-quinolinylnyl ester hydrochloride		Iv.	Mice	150	LD ₈₀
AR-18 T-(?)	Carbamic acid, N-methyl-8-quinolinylnyl ester methiodide		Iv. Iv. Sc. Sc. (In buffer solution)	Mice Mice Mice Mice	0.1 10 90 31	LD ₈₀ LD ₅₀ LD ₅₀ LD ₅₀
AR-38	Carbamic acid, N,N-dimethyl-8-quinolinylnyl ester methosulfate		Iv.	Mice	0.5	LD ₈₀
T-1972	Carbamic acid, N-methyl-1-methyl-1,2,3,4-tetrahydro-7-quinolinylnyl ester hydrochloride		?	Mice	30	LD ₅₀
T-1973	Carbamic acid, N-methyl-1-methyl-1,2,3,4-tetrahydro-7-quinolinylnyl ester methiodide		?	Mice	0.33	LD ₅₀

TABLE 2, Section XV (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-1937	Carbamic acid, N-methyl-1-methyl-1,2,3,4-tetrahydro-8-quinoliny ester methiodide		?	Mice	Approx. 45	LD ₅₀
T-1970	Isoquinoline, 2-methyl-1,2,3,4-tetrahydro-5,6-bis-(N-methylcarbamyloxy) hydrochloride		?	Mice	20	LD ₅₀
T-1971	Isoquinoline, 2-methyl-1,2,3,4-tetrahydro-5,6-bis-(N-methylcarbamyloxy) methiodide		?	Mice	60	LD ₅₀
T-1968	Isoquinoline, 2-methyl-1,2,3,4-tetrahydro-6,7-bis(N-methylcarbamyloxy) hydrochloride		?	Mice	Approx. 400-800	LD ₅₀
T-1969	Isoquinoline, 2-methyl-1,2,3,4-tetrahydro-6,7-bis(N-methylcarbamyloxy) methiodide		?	Mice	> 800	LD ₅₀

XVI. Carbamates of aliphatic alcohol derivatives.

TL-1251	Carbamic acid, 2-(dibutylamino)-ethyl ester butiodide	$I(C_4H_9)_3NCH_2CH_2OCONH_2$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1224	Carbamic acid, N-methyl-2-(dibutylamino)-ethyl ester butiodide	$I(C_4H_9)_3NCH_2CH_2OCONHCH_3$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1234	Carbamic acid, 2-(diethylamino)-ethyl ester ethiodide	$I(C_2H_5)_3NCH_2CH_2OCONH_2$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1152	Carbamic acid, N,N-dimethyl-2-diethylamino-ethyl ester ethiodide	$I(C_2H_5)_3NCH_2CH_2OCON(CH_3)_2$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1151	Carbamic acid, N-methyl-2-diethylaminoethyl ester ethiodide	$I(C_2H_5)_3NCH_2CH_2OCONHCH_3$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1154	Carbamic acid, N-methyl-2-piperidylethyl ester methiodide		Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1235	Carbamic acid, 3-(dibutylamino)-propyl ester butiodide	$I(C_4H_9)_3NCH_2CH_2CH_2OCONH_2$	Sc.W	Mice	80 40 20	0/2 0/2 0/2
TL-1225	Carbamic acid, N-methyl-3-(dibutylamino)-propyl ester butiodide	$I(C_4H_9)_3NCH_2CH_2CH_2OCONHCH_3$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1215	Carbamic acid, N-methyl-3-(diamylamino)-propyl ester amyliodide	$I(C_5H_{11})_3NCH_2CH_2CH_2OCONHCH_3$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2

TABLE 2, Section XVI (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1252	Carbamic acid, 2-(diamyl-amino)-propyl ester amyliodide	$\begin{array}{c} \text{CH}_3 \\ \\ \text{I}(\text{C}_5\text{H}_{11})_3\text{NCHCH}_2\text{OCONH}_2 \end{array}$	Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1399	Propane, 1-diethylamino-2,3-bis(N-methylcarbamyloxy)-, methiodide	$\begin{array}{c} \text{I} \\ \text{CH}_3(\text{C}_2\text{H}_5)_2\text{NCH}_2\text{CHCH}_2\text{OCONHCH}_3 \\ \\ \text{OCONHCH}_3 \end{array}$	Sc W.	Mice	80 40 20	0/2 0/2 0/2
TL-1514	Hexyne, 2,5-bis(N-methylcarbamyloxy)-high melting form	$\begin{array}{c} \text{C}-\text{CH}(\text{CH}_3)\text{OCONHCH}_3 \\ \\ \text{C}-\text{CH}(\text{CH}_3)\text{OCONHCH}_3 \end{array}$	Sc.P.	Mice	80 40 20	0/2 0/2 0/2
TL-1515	Hexyne, 2,5-bis(N-methylcarbamyloxy)-low melting form	$\begin{array}{c} \text{C}-\text{CH}(\text{CH}_3)\text{OCONHCH}_3 \\ \\ \text{C}-\text{CH}(\text{CH}_3)\text{OCONHCH}_3 \end{array}$	Sc.P.	Mice	80 40 20	0/2 0/2 0/2
T-(?)	Carbamic acid, N-benzyl-2-dimethylaminoethyl ester methiodide	$\text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCONHCH}_2\text{ } \langle \text{hexagon} \rangle$	Sc.	Mice	6.25	LD_{50}
T-(?)	Carbamic acid, N,N-di-benzyl-2-dimethylaminoethyl ester methiodide	$\begin{array}{c} \text{CH}_2\text{ } \langle \text{hexagon} \rangle \\ \\ \text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCON} \\ \\ \text{CH}_2\text{ } \langle \text{hexagon} \rangle \end{array}$	Sc.	Mice	75	LD_{50}
T-(?)	Carbamic acid, 3-dimethylaminopropyl ester methiodide	$\text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{CH}_2\text{OCONH}_2$	Sc.	Mice	37.5	LD_{50}
T-(?)	Carbamic acid, 4-dimethylaminobutyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{N}(\text{CH}_2)_3\text{CH}_2\text{OCONH}_2$	Sc.	Mice	12.5	LD_{50}
T-(?)	Carbamic acid, 10-dimethylaminodecyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{N}(\text{CH}_2)_9\text{CH}_2\text{OCONH}_2$	Sc.	Mice	75	LD_{50}
T-1096	Carbamic acid, 5-dimethylaminoamyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{N}(\text{CH}_2)_4\text{CH}_2\text{OCONH}_2$	Sc.	Mice	20	LD_{50}
T-1124	Carbamic acid, N-methyl-4-dimethylaminobenzyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{N} \langle \text{hexagon} \rangle \text{CH}_2\text{OCONHCH}_3$	Sc.	Mice	79	LD_{50}
T-(?)	Carbamic acid, N-methyl-2-dimethylaminoethyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCONHCH}_3$	Sc.	Mice	15	LD_{50}
T-(?)	Carbamic acid, N,N-dimethyl-2-dimethylaminoethyl ester methiodide	$\text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCON}(\text{CH}_3)_2$	Sc.	Mice	20	LD_{50}
T-(?)	Carbamic acid, N-ethyl-2-dimethylaminoethyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCONHC}_2\text{H}_5$	Sc.	Mice	60	LD_{50}
T-(?)	Carbamic acid, N,N-diethyl-2-dimethylaminoethyl ester methiodide	$\text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCON}(\text{C}_2\text{H}_5)_2$	Sc.	Mice	42.5	LD_{50}

TABLE 2, Section XVI (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
T-(?)	Carbamic acid, N,N-pentamethylene-2-dimethylaminoethyl ester methiodide	$\text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCON}$	Sc.	Mice	4	LD_{50}
T-(?)	Carbamic acid, N-allyl-2-dimethylaminoethyl ester methochloride	$\text{Cl}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCONHCH}_2\text{CH}=\text{CH}_2$	Sc.	Mice	37.5	LD_{50}
T-(?)	Carbamic acid, N-phenyl-2-dimethylaminoethyl ester methiodide	$\text{I}(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCONHC}_6\text{H}_5$	Sc.	Mice	450	LD_{50}
T-1093	Morpholine, N-(β-carbamoyloxyethyl)-, methochloride	$\text{CH}_3-\text{N}-\text{CH}_2\text{CH}_2\text{OCONH}_2$	Sc.	Mice	175	LD_{50}
XVII. Miscellaneous Carbamates.						
TL-1380	Physostigmine salicylate	CH_3NHCOO	Sc.W.	Mice Rats G. pigs Rabbits Cats	0.370 1.500 1.500 1.500 1.200 1.000 0.800 1.400 1.200 1.000	LD_{50} 0/2 0/2 0/2 2/2 2/2 2/2 1/2 1/2 0/2
AR-44	Physostigmine salicylate		Iv.	Mice	0.5	LD_{80}
AR-45	Physostigmine methiodide		Iv.	Mice	0.75-1.0	LD_{80}
TL-1400	Ammonium compound, substituted dimethyl-[β-(N-methylcarbamoyloxy)-γ-(3,4-methylenedioxyphenyl)propyl] (3,4-methylenedioxybenzyl) iodide	CH_3NHCOCH	Sc.P.	Mice	80 40 20	0/2 0/2 0/2

TABLE 2, Section XVII (Continued)

Code	Name	Structure	Route and solvent	Species	Dose mg/kg	Effect
TL-1411	Carbamic acid, N-methyl-3-dimethylamino- <i>d</i> -bornyl ester methiodide		Sc.W.	Mice	80 40 20	0/2 0/2 0/2
AR-43	Carbamic acid, N-methyl ester of Harmol hydrochloride		Iv.	Mice	66	LD ₈₀
SB-25	Carbamic acid, N,N-dimethyl-β-pyridyl ester hydrochloride		Sc.	Mice	120	LD ₅₀
XVIII. Carbamides and carbazates.						
TL-1517	Carbazic acid, 2,2-dimethyl-5-dimethylamino-2-methylphenyl ester dimethiodide		Sc.W.	Mice	80 40 20	0/2 0/2 0/2
TL-1516	Carbazic acid, 2,2-dimethyl-5-dimethylamino-2-methylphenyl ester dihydrochloride		Sc.W.	Mice	80 40 20	0/2 0/2 0/2
AR-26	Carbazic acid, 2-phenyl-3-dimethylaminophenyl ester methiodide		Iv.	Mice	0.25	LD ₅₀
TL-1402	Urea, 1-(4-hydroxy-2,3,5-trimethylphenyl)-3-methyl-		Sc.P.	Mice	80 40 20	0/2 0/2 0/2
TL-1401	Benzene, 1,4-bis(1,3-dimethylureido)-		Sc.W.	Mice	80 40 20	0/2 0/2 0/2

MISCELLANEOUS COMPOUNDS PREPARED OR EXAMINED AS CANDIDATE CHEMICAL WARFARE AGENTS

By *Marshall Gates*

14.1

INTRODUCTION

IN TABLE 1 of this chapter are grouped all those compounds which for one reason or another have not been subjected to detailed toxicological examination. With the average example, these substances showed insufficient toxicity to be seriously considered as chemical warfare agents, although other considerations, such as limited availability, instability, or lack of means for tactical employment have influenced decisions to abandon exploration of some compounds or the classes to which they belong.

Several of the compounds included or classes covered have been treated in other chapters of this volume. For example, cadmium, cadmium oxide, other cadmium compounds, some selenium derivatives, and several metallic carbonyls form the subject of Chapter 11. The tabulation of this chapter is intended to supplement such chapters by including references to the preparation and screening of the less promising members of such classes for the sake of completeness.

Although a number of compounds examined by the British have been included in the tabulation, no attempt has been made to give comprehensive coverage to British screening tests, since such systematic lists are provided elsewhere.⁵³

Perhaps worthy of mention in passing is the substance dichloroformoxime ("phosgene oxime"). It was examined in this country because intelligence reports and published literature indicated that some attention had been paid to it by the Germans and perhaps by the Russians. Dichloroformoxime possesses marked irritating action against skin which is manifested by an immediate burning sensation and the production of blisters. For this reason, the substance has been proposed as a "nettle" gas, but its limited stability, relatively low toxicity, and difficult preparation preclude serious consideration of it as a chemical warfare agent.

Dichloroformoxime exists when pure as a colorless solid of mp 39–40 C. It boils at 129 C without decom-

position at atmospheric pressure⁶³ and at 47–49 C at 23 mm^{42a} and is soluble in water and in organic solvents. It is rapidly destroyed by alkalis and is slowly hydrolyzed by water.⁶³ It possesses a penetrating and unpleasant odor and attacks the mucous membranes and the eyes severely.⁶³ The substance appears to be reasonably stable when pure and kept from contact with moisture^{56c,63} or when stored in anhydrous ether solution⁶⁰ but crude material rapidly decomposes on standing.^{44c}

Three distinct methods of preparation are described in the open literature:

1. The reduction of trichloronitrosomethane by hydrogen sulfide or aluminum amalgam,⁶⁴
2. The action of chlorine on fulminic acid⁶¹ or on mercury fulminate,⁶²
3. The chlorination of chloroisnitrosoacetone.⁶⁶

The first and third of these methods have been briefly examined by investigators under Division 9 of the National Defense Research Committee [NDRC] with disappointing results.^{42a,44b} The first gave rise to unspecified yields of material of poor quality which decomposed extensively in less than a day; the second gave only 30–40 per cent yields of crude material. The material which was examined physiologically by the University of Chicago Toxicity Laboratory melted below 35 C, and it is doubtful whether a pure sample of dichloroformoxime has been prepared or examined in this country.

The chlorination of fulminic acid salts has been investigated briefly in England.^{56c} The yields obtained (24–45 per cent) did not approach those claimed by Birckenbach and Sennewald.⁶¹ It was found that twice recrystallized material is considerably more stable than distilled material and can be stored for several weeks without undergoing appreciable decomposition.

The related dibromoformoxime has also been prepared and screened for toxicity.^{55b} It is less toxic than the prototype.

TABLE 1. Miscellaneous compounds prepared or examined as candidate chemical warfare agents.

The compounds in Table 1 are arranged in two large groups: (1) derivatives of heavy metals; and (2) miscellaneous organic compounds. Within the heavy metals group, the compounds are classified according to the periodic group of the metal, and, among each group of the periodic table, according to increasing atomic number. The miscellaneous organic compounds have been arranged according to the Beilstein system.

The following abbreviations are used: n_D^t , refractive index at t C; $d_{t_2}^{t_1}$, specific gravity at t_1 C in reference to water at t_2 C; mp, melting point in C; bp^p, boiling point in C at p mm Hg; vp^t, vapor pressure in mm Hg at t C; vol^t, saturation concentration (volatility) in mg/l at t C; and dec. p., decomposition point.

British reports concerned with those compounds marked by an asterisk are not all available in this country.

Centigrade scale is used throughout the table.

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
1. Cupric fluoroacetate	52	...	...	51
2. Cupric 2,4-dinitrobenzenearsonate	40a	...	...	...
3. Cupric 2,4,6-trinitrobenzenearsonate	40a	...	...	...
4. Silver nitrate	Commercial	...	...	24
5. Zinc fluoborate	40r	...	...	24
6. Zinc fluosilicate	40q	...	...	24
7. Strontium fluoborate	40r	...	...	24
8. Strontium fluosilicate	40r	...	...	24
9. Cadmium†	Commercial	...	...	See Chap. 11
10. Cadmium fluoride†	40o	...	...	24
11. Cadmium chloride†	Commercial	...	...	24
12. Cadmium nitrate†	Commercial	...	...	24
13. Cadmium oxide†	...	...	...	24
14. Cadmium sulfide†	Commercial	...	...	24
15. Cadmium selenide	22	...	...	24
16. Cadmium selenite	40q	...	...	24
17. Cadmium selenate†	22	...	...	24
18. Cadmium phosphite	...	...	...	24
19. Cadmium phosphate†	...	...	...	24
20. Cadmium fluoborate†	40p	...	...	24
21. Cadmium fluosilicate†	40q	dec.p. Approx. 100°	40q	24
22. Cadmium lactate	6	...	...	...
23. Cadmium butyrate	...	...	...	...
24. Cadmium caproate	6	...	...	...
25. Cadmium palmitate	6	...	...	...
26. Cadmium oleate	6	...	...	...
27. Cadmium stearate	6	...	...	...
28. Cadmium naphthenate	6	...	...	...
29. Cadmium oxalate	6	...	...	...
30. Cadmium malonate	6	...	...	...
31. Cadmium maleate	6	...	...	...
32. Cadmium fumarate	6	...	...	...
33. Cadmium succinate	6	...	...	...
34. Cadmium malate	6	...	...	...
35. Cadmium tartrate	6	...	...	...
36. Cadmium glutarate	6	...	...	...
37. Cadmium adipate	6	...	...	...
38. Cadmium mucate	6	...	...	...
39. Cadmium citrate	6	...	...	...
40. Cadmium chelate of acetylacetone	6	dec.p. 280–285°	6	...
41. Cadmium enolate of ethyl nitromalonate	6	...	...	...
42. Cadmium salt of nitrated oxidized starch	Commercial	...	...	24
43. Cadmium salt of 2,4-dinitrophenol	6	...	...	...
44. Cadmium picrate	6	dec.p. 250°	6	...
45. Cadmium chelate of dinitroresorcinol	6	...	...	...
46. Cadmium styphnate	6	...	...	...
47. Cadmium <i>m</i> -nitrobenzenesulfonate	6	...	...	...
48. Cadmium 2,4-dinitrobenzenesulfonate	6	...	...	...
49. Cadmium <i>p</i> -nitrobenzoate	6	...	...	...
50. Cadmium 2,4-dinitrobenzoate	6	...	...	...
51. Cadmium 3,5-dinitrobenzoate	6	...	...	...

† These compounds are discussed more fully in Chapter 11.

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
52. Cadmium 2,4,6-trinitrobenzoate	6	...	...	...
53. Cadmium chelate of salicylaldehyde	6	mp	>300°	6
54. Cadmium chelate of salicylaldoxime	6	mp	>300°	6
		dec.p.	280°-290°	6
55. Cadmium salicylate	6	...	...	...
56. Cadmium 3-nitrosalicylate	6	...	...	...
57. Cadmium 5-nitrosalicylate	6	...	...	...
58. Cadmium phthalate	40k	...	...	...
59. Cadmium <i>o</i> -nitrocinnamate	6	...	...	...
60. Cadmium <i>m</i> -nitrocinnamate	6	...	...	...
61. Cadmium <i>p</i> -nitrocinnamate	6	...	...	...
62. Cadmium salt of hexanitrodiphenylamine	6	...	...	...
63. Cadmium <i>o</i> -nitrobenzenearsonate	6	...	...	...
64. Cadmium 2,4-dinitrobenzenearsonate	6	...	...	...
65. Cadmium 2,4,6-trinitrobenzenearsonate	6	...	...	...
66. Cadmium 3,5-dinitro-4-hydroxybenzenearsonate	6	...	...	...
67. Cadmium 3,5-dinitro-2,4-dihydroxybenzene- arsonate	6	...	...	...
68. Cadmium furoate	6	...	...	...
69. Cadmium 5-nitro-2-furoate	6	...	...	...
70. Cadmium dehydromucate	6	...	...	...
71. Cadmium 5-nitro-2-furylacrylate	6	...	...	...
72. Cadmium chelate of 8-hydroxyquinoline	6	mp	>325°	6
73. Dimethylcadmium	6	bp	98-99°	6
74. Diethylcadmium	6	bp ²⁰	62-64°	6
75. Dipropylcadmium	6	bp ⁵	67°	6
76. Barium fluoborate	40p	mp	>200°	40p
77. Barium fluosilicate	40p	mp	>200°	40p
78. Barium succinate	...	...	...	...
79. Barium salt of 2,4-dinitrophenol	40e	...	...	...
80. Barium 3,5-dinitrobenzoate	40e	...	...	...
81. Barium 2,4,6-trinitrobenzoate	40e	...	...	...
82. Barium salt of dipicrylamine	40e	...	...	...
83. Barium 2,4-dinitrobenzenearsonate	40a	...	...	...
84. Barium 2,4,6-trinitrobenzenearsonate	40a	...	...	...
85. Barium 5-nitro-2-furoate	40e	...	...	24
86. Barium 5-nitro-2-furylacrylate	40e	...	...	...
87. Mercuric chloride	Commercial	...	...	24
88. Mercuric fluoroacetate	52	...	...	51
89. Mercury salt of nitrated oxidized starch	Commercial	...	...	24
90. Mercuric 2,4-dinitrobenzenearsonate	40a	...	...	...
91. Mercuric 2,4,6-trinitrobenzenearsonate	40a	...	...	...
92. Chlorovinylmercuric chloride	40j	...	...	24, 33
93. Butylmercuric iodide	40e	...	...	...
94. Butylmercuric hydroxide	40e	...	...	...
95. 2-Chloromercurifuran	28	mp	151-152.5°	28
96. 2,5-(Dichloromercuri)furan	28	...	...	24
97. 2-Chloromercurithiophene*	28	mp	183-184°	28
98. 2,5-bis(Chloromercuri)thiophene*	...	...	...	...
99. Difurylmercury	40e	...	...	24, 33
100. Thallous fluoride	15	bp	298°	15
	...	mp	288°	15
101. Thallous fluoborate	15	bp ⁸	300°	15
102. Thallous selenite	40r	...	...	...
103. Thallous fluosilicate	15	bp ⁸	340°	15
104. Thallous ethoxide	15	...	...	...
105. Thallous β -chloroethylmercaptide	40p	mp	>300°	40p
106. Thallous formate	15	...	...	...
107. Thallous acetate	15	...	...	24
108. Thallous fluoroacetate	52	...	...	51
109. Thallous trifluoroacetate	40p	mp	116-119°	40p
110. Thallous salt of ethyl nitromalonate	40c	...	...	...

SECRET

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
111. Thallium salt of nitrated oxidized starch	Commercial	...	...	24
112. Thallous benzoate	15	...	...	...
113. Thallous <i>p</i> -nitrobenzoate	15	...	...	...
114. Thallous 2,4-dinitrobenzoate	15	...	...	...
115. Thallous 3,5-dinitrobenzoate	15	...	...	...
116. Thallous <i>m</i> -trifluoromethylbenzoate	40p	...	...	24
117. Thallous salt of 2,4,6,2',4',6'-hexanitrodiphenylamine	15	...	...	...
118. Thallous furoate	15	...	...	...
119. Thallous 5-nitro-2-furoate	15	...	...	...
120. Thallous 5-nitro-2-furylacrylate	15	...	...	...
121. Thallous N-methyldithiocarbamate	15	...	...	...
122. Thallous N,N-dimethyldithiocarbamate	15	mp	124–125°	15
123. Thallous N-ethyldithiocarbamate	15	...	...	...
124. Thallous N-isopropyldithiocarbamate	15	...	...	...
125. Thallous N,N-diethyldithiocarbamate	15	bp ^{0.01–.02}	190°	15
	...	mp	110–111°	15
126. Thallous N-butyldithiocarbamate	15	...	...	...
127. Thallous N,N-diisopropyldithiocarbamate	15	...	...	...
128. Thallous N-cyclohexyldithiocarbamate	15	...	...	...
129. Thallous N,N-dibutyldithiocarbamate	15	bp ^{0.01–.02}	230–235°	15
	...	mp	75–77°	15
130. Thallous N,N-diisobutyldithiocarbamate	15	mp	165–165.5°	15
131. Dimethylthallium fluoride	15	...	...	...
132. Dimethylthallium iodide	15	...	...	...
133. Dimethylthallium hydroxide	15	...	...	...
134. Dimethylthallium fluoborate	15	mp	303°	15
135. Dimethylthallium fluosilicate	15	mp	>300°	15
136. Dimethylthallium ethoxide	15	...	...	24
137. Dimethylthallium ethylmercaptide	...	...	...	24, 33
138. N-Dimethylthallium dimethylamine	40i	...	...	...
139. N-Dimethylthallium diethylamine	40i	...	...	...
140. N-Dimethylthallium dibutylamine	40i	...	...	...
141. Dimethylthallium acetylacetone	15	mp	214–215°	15
142. Dimethylthallium ethyl acetoacetate	15	mp	128–130°	15
143. Dimethylthallium trifluorohexoylacetone	...	...	...	24, 33
144. N-Dimethylthallium methylaniline	40i	...	...	...
145. Dimethylthallium salicylaldehyde	15	mp	200°d	15
146. Dimethylthallium N,N-diethyldithiocarbamate	15	bp ¹	130°	15
	...	bp ⁴	138°	15
147. Dimethylthallium N,N-diisopropyldithiocarbamate	15	bp ¹	130°	15
	...	bp ^{5.5}	145°	15
	...	mp	150°	15
148. Dimethylthallium N,N-dibutyldithiocarbamate	15	bp ^{0.5}	147–148°	15
149. Dimethylthallium N,N-diisobutyldithiocarbamate	15	bp ^{0.5}	104–105°	15
	...	mp	73–74°	15
150. Diethylthallium bromide	15	...	...	...
151. Diethylthallium ethoxide	15	...	...	...
152. Diethylthallium trifluoroacetate	15	mp	233–235°	15
153. Diethylthallium acetylacetone	15	dec.p.	240°	15
154. Diethylthallium benzoylacetone	40d	...	...	...
155. Diethylthallium thioacetate	15	mp	181–183°	15
156. Dipropylthallium ethoxide	15	...	...	...
157. Dipropylthallium- <i>d</i> -camphor-10-sulfonate	15	...	...	15
158. Diisopropylthallium chloride	15	mp	150°d	15
159. Dibutylthallium fluoride	15	mp	220–230°	15
160. Dibutylthallium chloride	15	mp	240–245°	15
161. Dibutylthallium bromide	15	mp	245–250°	15
162. Dibutylthallium iodide	15	mp	220–225°	15
163. Diisoamylthallium acetylacetone	40d	...	...	...
164. Diphenylthallium chloride	40d	...	...	...

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties			Refer. to toxicity data
		Property	Reference		
165. Diphenylthallium iodide	15	mp	>300°	15	...
166. Diphenylthallium hydroxide	40d	...		...	...
167. Difurylthallium fluoride	15	mp	235–240°d	15	...
168. Difurylthallium iodide	15	mp	238–240°	15	...
169. Tetramethylgermanium	...	...		...	24
170. Stannic 2,4-dinitrobenzenearsonate	40a	...		...	...
171. Stannic 2,4,6-trinitrobenzenearsonate	40a	...		...	...
172. Butyltin triiodide	2	bp ⁵	154°	2	2
173. Dipropyltin dibromide	2	bp ^{0.3}	112°	2	2
	...	mp	49–50°	2	2
174. Dibutyltin diiodide	2	bp ^{5.5}	157°	2	2
175. Di- <i>tert</i> -butyltin dibromide	2	bp ¹⁴	128°	2	2
176. <i>bis</i> (2-Pyridyl)tin bromide	40f	...		...	...
177. Trimethyltin bromide	2	bp ⁸	46–47°	2	2
178. Trimethyltin hydroxide	2	sublim.p.	105–108°	2	...
179. Triethyltin hydride	2	bp ⁸	36°	2	...
180. Triethyltin bromide	2	bp	216–217°	2	2, 24
181. Tripropyltin hydride	2	bp ²	65°	2	24
	...	<i>d</i> ₄ ²⁵	1.1452	2	...
182. Tripropyltin bromide	2	bp ⁸	123°	2	2, 24
183. Triisopropyltin bromide	2	bp ³	79°	2	...
184. Triisopropyltin iodide	2	bp ⁴	108–110°	2	2
185. Tributyltin hydride	2	bp ⁸	115°	2	24
	...	<i>d</i> ₄ ²⁵	1.108	2	...
186. Tributyltin chloride	2	bp ^{1.5}	119°	2	2
	...	<i>d</i> ₄ ²⁶	1.134	2	...
187. Tributyltin bromide	2	bp ³	156°	2	2, 24, 33
188. Tributyltin iodide	2	bp ²	138–139°	2	2, 24, 33
	...	<i>d</i> ₄ ²⁶	1.501	2	...
189. Tributyltin cyanide	2	mp	68°	2	...
190. Tributyltin thiocyanate	2	bp ^{0.5}	160°	2	2
191. Tributyltin hydroxide	2	<i>d</i> ₄ ²⁵	1.160	2	2
192. Tributylethoxytin	2	bp ^{0.5}	105°	2	2, 24
	...	<i>d</i> ₄ ²⁷	1.101	2	...
193. Tributylthioethoxytin	2	bp ^{1.5}	126°	2	...
	...	<i>d</i> ₄ ²⁶	1.132	2	...
194. Triamyltin bromide	2	bp ^{1.5}	162°	2	2
195. Triisoamyltin bromide	2	bp ³	135°	2	...
196. Triisoamyltin iodide	2	bp ¹	140–142°	2	...
197. Trihexyltin bromide	2	bp ²	194°	2	2
198. Triphenyltinbenzenesulfonamide	56b	mp	119°	56b	56b
199. Tetramethyltin	2	...		...	24
200. Tetraethyltin	2	bp	176–180°	2	...
201. Tetrapropyltin	2	bp ¹⁰	112°	2	...
202. Tetraisopropyltin	2	bp ⁹	116°	2	...
203. Tetrabutyltin	2	...		...	...
204. Tetraamyltin	2	bp ¹⁰	181°	2	...
205. Tetraisoamyltin	2	bp ²⁴	188°	2	...
206. Tetrahexyltin	2	bp ¹⁰	209°	2	...
207. Tetradecyltin	2	...		...	...
208. Lead fluosilicate	40q	mp	>200°	40q	24
209. Lead salt of nitromethane	1	...		...	...
210. Lead salt of nitroaminoguanidine	1	...		...	...
211. Lead salt of dinitrotartaric acid	1	...		...	...
212. Lead- <i>m</i> -nitrobenzenesulfonate	1	...		...	...
213. Lead 2,4-dinitrobenzenesulfonate	1	...		...	...
214. Lead benzoate	1	...		...	...
215. Lead <i>o</i> -nitrobenzoate	1	...		...	...
216. Lead <i>m</i> -nitrobenzoate	1	...		...	...
217. Lead <i>p</i> -nitrobenzoate	1	...		...	...
218. Lead 2,4-dinitrobenzoate	1	...		...	...
219. Lead 3,5-dinitrobenzoate	1	...		...	...

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
220. Lead 2,4,6-trinitrobenzoate	1	...	...	...
221. Lead salt of <i>p</i> -nitrophenylhydroxamic acid	1	...	...	...
222. Lead salt of <i>m</i> -phenylenedinitroamine	1	...	...	...
223. Lead <i>o</i> -nitrobenzenearsonate	1	...	...	...
224. Lead <i>m</i> -nitrobenzenearsonate	1	...	...	...
225. Lead 2,4-dinitrobenzenearsonate	...	...	...	...
226. Lead 2,4,6-trinitrobenzenearsonate	1	...	...	...
227. Lead 3-nitro-4-hydroxybenzenearsonate	1	...	...	...
228. Lead 3,5-dinitro-4-hydroxybenzenearsonate	1	...	...	...
229. Lead 3,5-dinitro-4-aminobenzenearsonate	1	...	...	...
230. Lead 5-nitrofuroate	1	...	...	...
231. Lead 5-nitrofurylacrylate	1	...	...	...
232. Diethyllead dinitrate	1	...	...	...
233. Diethyllead selenite	16	mp	>286°	16
234. Diethyllead <i>bis</i> (<i>p</i> -chlorobenzoate)	16	mp	185°d	16
235. Diethyllead <i>bis</i> (<i>m</i> -bromobenzoate)	16	mp	178–179°d	16
236. Diethyllead <i>bis</i> (<i>m</i> -nitrobenzoate)	16	mp	179–180°d	16
237. Diethyllead <i>bis</i> (<i>p</i> -toluate)	16	mp	186°d	16
238. Diethyllead <i>bis</i> (<i>N</i> -butylanthranilate)	16	mp	169–169.5°d	16
239. Diethyllead dinicotinate	16	mp	143°d	16
240. Diethyllead dithioacetate*	56a	mp	84.5–85°	56a
241. Dibutyllead dinitrate	1	...	...	...
242. Diphenyllead dinitrate	1	...	...	...
243. <i>bis</i> (<i>m</i> -Nitrophenyl)lead dichloride	1	...	...	...
244. <i>bis</i> (<i>m</i> -Nitrophenyl)lead dibromide	1	...	...	...
245. <i>bis</i> (<i>m</i> -Nitrophenyl)lead diiodide	1	...	...	...
246. <i>bis</i> (<i>m</i> -Nitrophenyl)lead dinitrate	1	...	...	...
247. <i>bis</i> (<i>m</i> -Nitrophenyl)lead oxide	1	...	...	...
248. Trimethyllead <i>p</i> -toluenesulfonate*	...	...	...	...
249. Triethyllead thiocyanate*	16	mp	26.5–27°	16
250. Triethyllead selenocyanate*	16	mp	33–34°	16
251. Triethyllead nitrate	1	...	...	...
252. <i>bis</i> (Triethyllead) fluosilicate	...	...	...	24
253. Triethyl- β -chlorothioethoxylead	40m	...	...	24
254. Triethyllead fluoroacetate	56e	mp	180.5°	56e
255. Triethyllead α -chlorocrotonate	16	mp	153–155°	16
256. Triethyllead acid oxalate	16	mp	>300°	16
257. <i>bis</i> (Triethyllead) oxalate	16	mp	>300°	16
258. <i>bis</i> (Triethyllead) fumarate	16	dec.p.	165°	16
259. <i>bis</i> (Triethyllead) adipate	16	mp	>360°	16
260. <i>bis</i> (Triethyllead) <i>d</i> -camphorate	16	mp	>310°	16
261. <i>tris</i> (Triethyllead) citrate	16	mp	>350°	16
262. Triethyllead <i>m</i> -chlorobenzoate	40d	...	...	...
263. Triethyllead <i>p</i> -chlorobenzoate	16	mp	123–124°	16
264. Triethyllead <i>o</i> -bromobenzoate	16	mp	134–135°	16
265. Triethyllead <i>m</i> -bromobenzoate	16	mp	113–114°	16
266. Triethyllead <i>p</i> -bromobenzoate	16	mp	127–128°	16
267. Triethyllead <i>o</i> -iodobenzoate	16	mp	138.5–139°	16
268. Triethyllead <i>m</i> -iodobenzoate	16	mp	135–136°	16
269. Triethyllead <i>p</i> -iodobenzoate	16	mp	129.5–130.5°	16
270. Triethyllead <i>o</i> -nitrobenzoate	16	mp	142–143°d	16
271. Triethyllead <i>m</i> -nitrobenzoate	16	mp	172–173°d	16
272. Triethyllead <i>p</i> -nitrobenzoate	16	mp	167–168.5°d	16
273. Triethyllead salicylate	16	mp	75–76°	16
274. Triethyllead <i>p</i> -anisate	16	mp	97–98°	16
275. Triethyllead <i>p</i> -aminobenzoate	16	dec.p.	265°	16
276. Triethyllead <i>p</i> -aminobenzoate monohydrate	16	mp	84–86°	16
277. Triethyllead <i>N</i> -methylantranilate	16	mp	132.7°d	16
278. Triethyllead <i>N</i> -phenylantranilate	16	mp	124.5–125°	16
279. Triethyllead phenylacetate	16	mp	96–97°	16
280. Triethyllead <i>p</i> -aminophenylacetate	40d	...	...	...
281. Triethyllead phenylpropiolate	16	mp	149–150°d	16

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
282. Triethyllead cinnamate	16	mp	122–123°d	16
283. Triethyllead β -benzoylacrylate	16	mp	139–140°d	16
284. Triethyllead 9-fluorene-carboxylate	16	dec.p.	208°	16
285. Triethyllead β (2-naphthoyl)propionate	16	mp	134–135°	16
286. Triethyllead diphenylacetate	16	mp	164–165°	16
287. Triethyllead triphenylacetate	16	mp	134–136°d	16
288. Triethylleadsulfanilamide	56b	mp	171°	56b
289. Triethyllead furoate	16	mp	156–157°d	16
290. Triethyllead furylacrylate	16	mp	132–133°d	16
291. Triethyllead lepidine-2-carboxylate	16	mp	153–155°	16
	...	dec.p.	197–199°	16
292. Triethyllead N-ethylcarbazole-3-carboxylate	16	mp	195°d	16
293. Triethyllead thioacetate*	56a	mp	44°	56a
294. Triethyllead cyclohexylsulfinate	16	mp	132–134°	16
295. Triethyllead <i>p</i> -toluenesulfinate	16	mp	86–88°	16
296. Triethyllead <i>o</i> -toluenesulfonate*	56a	mp	87°	56a
297. Triethyllead <i>p</i> -toluenesulfonate*	...	...		...
298. Triethyllead 2-amino-5-toluenesulfonate	16	mp	210°d	16
299. Triethyllead naphthalene-2-sulfonate*	...	...		...
300. Triethyllead <i>d</i> -camphor-10-sulfonate	16	mp	172°	16
301. Triethyllead <i>p</i> -tolylthiosulfonate	16	mp	109°	16
302. Triethyllead methanesulfonamide*	56b	mp	97°	56b
303. Triethyllead methanesulfonanilide*	56b	mp	115.5°	56b
304. bis(Triethyllead) methanedisulfonate	56b	...		...
305. bis(Triethyllead) methanedisulfonanilide	56b	...		...
306. Triethyllead ethanesulfonanilide	56b	mp	110°	56b
307. Triethyllead benzenesulfonamide	...	...		...
308. Triethyllead <i>p</i> -aminobenzenesulfonamide	16, 56b	mp	173–174°	16
309. Triethyllead <i>o</i> -toluenesulfonamide*	56b	mp	133°	56b
310. Triethyllead <i>p</i> -toluenesulfonamide*	...	...		...
311. Triethyllead <i>p</i> -toluenesulfonanilide*	56b	mp	134°	56b
312. Triethyllead <i>p</i> -toluenesulfon- <i>p</i> -chloranilide	56b	mp	111.5°	56b
313. Triethyllead <i>p</i> -toluenesulfon- <i>p</i> -bromanilide	56b	mp	117°	56b
314. Triethyllead <i>o</i> -carboxybenzenesulfonimide*	56b	mp	135°	56b
315. Tripropyllead <i>o</i> -toluenesulfonate	56a	mp	87°	56a
316. Tripropyllead <i>p</i> -toluenesulfonate	56a	mp	73–74.5°	56a
317. Triethyllead 1-amino-4-naphthalenesulfonate	16	mp	238–240°	16
318. Tripropyllead methanesulfonamide*	56b	mp	67°	56b
319. Tripropyllead benzenesulfonamide	...	...		...
320. Tripropyllead <i>p</i> -aminobenzenesulfonamide	56b	mp	101°	56b
321. Tripropyllead <i>p</i> -toluenesulfonanilide	56b	mp	104°	56b
322. Tripropyllead <i>p</i> -toluenesulfon- <i>p</i> -chloranilide*	56b	mp	123°	56b
323. Tripropyllead <i>o</i> -carboxybenzenesulfonimide	56b	mp	130°	56b
324. Tributyllead <i>p</i> -toluenesulfonate	56a	mp	81–82°	56a
325. Tributyllead naphthalene-2-sulfonate	56a	mp	68°	56a
326. Triphenyllead nitrate	1	mp	220–225°	1
		(sinter)		
327. Tri(<i>m</i> -nitrophenyl)lead chloride	1	...		...
328. Tri(<i>m</i> -nitrophenyl)lead nitrate	1	...		...
329. Tetramethyllead	...	...		...
330. Triethylallyllead dimer	40h	...		...
331. Antimony trifluoride	Commercial	...		...
332. Ethyldichlorostibine	13	bp ¹	62–83°	13
	...	<i>d</i>	2.182	13
333. <i>p</i> -Thiocyanophenyldichlorostibine*	...	...		...
334. <i>p</i> -Ethylthiophenyldichlorostibine*	...	...		...
335. <i>p</i> -(β -Chloroethylthio)phenyl dichlorostibine*	...	...		...
336. <i>p</i> -Phenylene-sinestibine tetrachloride*	...	...		...
337. bis(<i>m</i> -Aminophenyl)chlorostibine dihydrochloride	...	...		...
338. bis(<i>m</i> -Aminophenyl)hydroxystibine	...	...		...
339. 5,10-Dichloro-5,10-dihydrostibarsanthrene*	...	...		...
340. Diphenyl- α -thienylstibine*	...	...		...

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
341. Phenylldithienylstibine	...	...	...	...
342. Trifurylantimony	40e	...	...	24, 33
343. <i>tris</i> (5- <i>tert</i> -Butyl-2-furyl)antimony	40f	...	...	24, 33
344. <i>tris</i> (2-Pyridyl)antimony	40g	...	...	24, 33
345. Trimethylstibine sulfide*	...	...	...	...
346. <i>bis</i> (Trimethylstibo)trisulfide*	...	...	...	...
347. <i>bis</i> (Diphenylstibine)sulfide*	...	...	...	...
348. Sulfate of <i>bis</i> (<i>m</i> -aminophenyl)hydroxystibine*	...	...	...	...
349. 5,10-Dihydro-5,10-dioxystibarsanthrene-5,10-monoxide	...	...	...	...
350. Diphenylbismuth thiocyanate*	...	...	...	56d
351. <i>tris</i> (2-Furyl)bismuth	40f	...	...	...
352. Chromyl chloride	28	bp	114°	24
	...	d_4^{25}	1.912	28
353. Chromium hexacarbonyl	40g	...	...	...
354. Chromium 5-nitro-2-furoate	40c	...	...	...
355. Tungsten carbonyl	36	mp	125°	35
	...	vp ⁶⁷	1.2	36
356. Manganous 2,4-dinitrobenzenearsonate	40a	...	...	...
357. Iron pentacarbonyl*	Commercial	...	...	See Chap. 11
358. Ferric 2,4-dinitrobenzenearsonate	40a	...	...	...
359. Cobaltous fluoborate	...	...	...	24
360. Salcomine	Commercial	...	...	35
361. Cobalt 2,4-dinitrobenzenearsonate	40a	...	...	...
362. Nickel carbonyl*	...	...	...	See Chap. 11
363. Nickel fluoborate	40q	...	...	24
364. Nickel fluosilicate	40r	mp	>275°	40r
365. Nickel 2,4-dinitrobenzenearsonate	40a	...	...	...
366. Chlorine	Commercial	...	...	24, 59
367. Bromine	Commercial	...	...	24, 59
368. Nitrogen fluoride	...	...	...	24
369. Ammonium fluoride	Commercial	...	...	24
370. Lithium hypochlorite	...	...	...	24
371. Hydrogen sulfide	...	mp	-85.5°	59
	...	bp	-60.3°	59
372. Sulfur monofluoride*	54, 57	bp	-35°	54
	...	...	-99°	...
	...	mp	-105.5°	54
	...	d^{-100}	1.5	54
373. Sulfur tetrafluoride*	54, 57	bp	-40°	54
	...	mp	-124°	54
374. Sulfur hexafluoride	...	...	...	24
375. Disulfurdecafluoride	See Chap. 4	...	...	See Chap. 4
376. Thionyl fluoride*	54, 57	bp	-43.8°	54
	...	mp	-129.5°	54
377. Sulfuryl fluoride*	54, 57	bp	-52°	54
	...	mp	-120°	54
378. Sulfuryl chlorofluoride	...	...	...	24
379. Pyrosulfuryl chloride	...	...	...	24
380. Hydrogen selenide	...	bp	-41.5°	59
381. Sodium selenide	Commercial	...	...	24
382. Selenium monochloride†	59	bp ⁷³³	127°	59
	...	d^{25}	2.7741	59
	...	n_D^{25}	1.5962	59
383. Selenium monobromide†	...	...	...	...
384. Selenium hexafluoride†	57	sublim.p.	-46.6°	54
385. Carbon sulfideselenide†	...	...	...	...
386. Carbon diselenide†	22	bp	117-118°	22
387. Selenium oxychloride†	23	bp ²¹	84-85°	23
	...	mp	10.9°	59
	...	n_D^{20}	1.6516	59

† These compounds and other selenium compounds are discussed more fully in Chapter 11.

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
388. Selenium oxybromide†	...	...	...	...
389. Selenium oxide	Commercial	...	...	24
390. Selenium dioxide†	...	...	...	...
391. Chloroselenious acid†	...	...	...	...
392. Sodium selenite	...	...	...	24
393. Hydrazine hydrate	Commercial	...	...	24
394. Ammonium fluosilicate	Commercial	...	...	24
395. Trichloronitrosomethane	7	...	...	24
396. Chloropicrin	Commercial	bp ⁷⁶⁰	112°	65
	...	fp	-69.2°	65
	...	vol ²⁰	167	26
397. Diiodoacetylene	27	mp	78-80°	27
398. Nitroethylene	Commercial	...	...	24, 33
399. 2-Propynyl chloride	27	bp	58-65°	27
	...	<i>n</i> _D ^{18.5}	1.4405	27
400. 1-Nitropropene	Commercial	vol ²⁰	18.66	26
401. 2-Nitropropene	Commercial	vol ²⁰	64.14	26
402. 1,2-Dichloro-2-nitrosopropane	7	bp ⁴¹	41-42°	7
	...	<i>n</i> _D ²⁵	1.4323	7
	...	<i>d</i> ₄ ²⁵	1.239	7
403. 1,1,1-Trichloro-3-nitro-2-propene	18	bp ¹	44-45°	18
	...	mp	-4.4°	18
	...	<i>n</i> _D ²⁰	1.5172	18
	...	<i>d</i> ²⁵	1.5562	18
404. 2-Bromo-2-nitrosopropane	49a	bp ¹⁰⁰	40°	49a
405. 2-Nitrobutene-1	...	vol ²⁰	32.36	26
406. 1,4-Dibromo-2-butene	7	mp	54°	7
407. <i>tris</i> (Chloromethyl)nitromethane	47a	...	...	...
408. 3-Chloro-3-nitrosopentane	7	bp ³⁸	44°	7
	...	<i>n</i> _D ²⁵	1.4190	7
	...	<i>d</i> ₄ ²⁵	1.016	7
409. Methyl sulfite	...	...	...	...
410. Methyl silicate	7	bp ¹⁵⁵	75°	7
411. Dimethyl selenide	22	bp	56-58°	22
412. Trimethylselenonium fluoride	20	<i>d</i> ³²	1.378	20
	...	<i>n</i> _D ²⁰	1.4600	20
413. Dimethyl telluride*	...	...	...	...
414. Monochloromethyl sulfate*	...	...	...	...
415. <i>bis</i> (Chloromethyl) sulfate*	...	...	...	...
416. <i>bis</i> (Chloromethyl) ether*	...	...	...	...
417. <i>bis</i> (Bromomethyl) ether	59, 40l	mp	-34°	59
	...	bp	154-155°	59
	...	<i>d</i> ²⁰	2.2013	59
418. β-Chlorovinylselenium chloride*	23	mp	86°	23
419. Ethyl sulfite	...	...	...	...
420. Ethyl fluorosulfonate	...	...	...	...
421. Ethyl chloroselenite*	...	...	...	...
422. Ethyl selenomercaptan*	...	...	...	...
423. Ethoxyselenyl chloride*	...	bp ¹⁵	81.5-82.5°	55e
424. Diethyl selenide*	22	bp ¹⁸	79-82°	22
425. Diethyl diselenide*	...	...	...	...
426. Diethyl telluride	...	...	...	...
427. <i>tris</i> (β-Chloroethyl) borate	40s	bp ¹	97-99°	40s
428. <i>tetrakis</i> (β-Chloroethyl) orthosilicate	21	bp ¹	142-143°	21
429. β-Chloroethyl nitrite	7	bp ⁸⁰	33°	7
	...	<i>n</i> _D ²⁰	1.4115	7
	...	<i>d</i> ²⁰	1.212	7
430. Methyl β-chloroethyl sulfite*	...	...	...	...
431. <i>bis</i> (β-Chloroethyl) sulfite*	...	...	...	...
432. <i>bis</i> (β-Chloroethyl) selenite*	...	mp	44-45°	55e
433. β-Chloroethylsulfuryl chloride*	12	bp ^{0.5}	60-64°	12

† These compounds and other selenium compounds are discussed more fully in Chapter 11.

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
434. <i>bis</i> (β -Chloroethyl) sulfate	12	bp ^{0.5}	117–133°	12
435. <i>bis</i> (β -Chloroethyl) ether	Commercial	...		...
436. <i>bis</i> (β -Chloroethyl) selenide*	22	bp ^{0.1}	96–100°	22
437. <i>bis</i> (β -Chloroethyl) selenium dichloride*	...	...		...
438. 2-Propyn-1-ol	27	bp	113–117°	27
	...	n_D^{20}	1.4330	27
439. 3-Bromo-2-propyn-1-ol	27	bp ²	49–53°	27
	...	n_D^{20}	1.5140	27
440. 3-Iodo-2-propyn-1-ol	27	bp ⁵	82–85°	27
	...	mp	40–43°	27
441. Methyl 2-propynyl ether	27	bp	61–65°	27
	...	n_D^{25}	1.4052	27
442. Methyl 3-bromopropynyl-2 ether	27	bp ¹⁶	34–38°	27
	...	n_D^{25}	1.4793	27
443. 3-Chloroallyl alcohol	...	...		...
444. Allyl methyl ether	...	...		...
445. <i>sym</i> -Dichloroisopropyl chlorosulfinate	32	bp ¹⁵	108–110°	32
	...	n_D^{20}	1.5130	32
	...	d^{20}	1.432	32
446. 2-Nitro-1-butanol silicate	40b	...		...
447. Ethinyldimethylvinyl carbinol	Commercial	...		...
448. 2-Butyne-1,4-diol	Commercial	...		...
449. <i>bis</i> - β -Chloroethyl formal	12	bp ¹²	92–94°	12
450. Methylformylchloride oxime	19	bp	65–66°	19
	...	mp	–64 to –60°	19
	...	n_D^{25}	1.4193	19
	...	d^{25}	1.135	19
451. Acetaldehyde azine	7	bp	96–98°	7
	...	n_D^{25}	1.4370	7
452. Hemiacetal of chloral and chloretone	47b	mp	68–69°	47b
453. Chloral oxime	7	bp ⁵⁹	69–70°	7
	...	n_D^{25}	1.4905	7
	...	d_4^{25}	1.571	7
454. Acrolein	Commercial	...		...
455. Propionaldehyde azine	7	bp	139–141°	7
	...	n_D^{25}	1.4497	7
456. Acetone azine	7	bp	129–133°	7
	...	n_D^{25}	1.4511	7
457. <i>bis</i> (Selenoacetone)*	...	...		...
458. Chloroacetone oxime	7	bp ⁶	70–71°	7
	...	n_D^{25}	1.4777	7
	...	d_4^{25}	1.221	7
459. Bromoacetone	21	bp ¹³	35.5–36.5°	21
460. Butyraldehyde azine	7	bp ¹⁷	77–78°	7
	...	n_D^{25}	1.4504	7
461. Methyl ethyl ketone azine	7	bp ²¹	71–72°	7
	...	n_D^{25}	1.4517	7
462. 1-Bromobutanone-2	21	bp ³⁰	62–66°	21
	...	n_D^{15}	1.4700	21
463. 3-Bromobutanone-2	21	bp ³⁰	49–53°	21
	...	n_D^{15}	1.4595	21
464. Selenoalvaleraldehyde*	...	...		...
465. Diethyl ketone azine	7	bp ²³	94–96°	7
	...	n_D^{25}	1.4539	7
466. 1-Bromopentanone-2	21	...		...
467. 3-Bromopentanone-2	21, 58	bp ¹⁵	75–76°	21
	...	n_D^{23}	1.4576	21
	...	vol ²⁰	21.59	26
468. α -Chloromesityl oxide	39	bp ³¹	66–69°	39
469. 1-Hydroxy-2-pentyne-4-one	27	bp ³	79–83°	27
	...	n_D^{20}	1.4587	27
	...	vol ²⁰	0.111	26

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data	
		Property	Reference		
470. 1-Methoxy-2-pentyne-4-one	27	bp ³	47–50°	27	24, 33
	...	<i>n</i> _D ²⁰	1.4462	27	...
471. Carbon suboxide	38	...		...	24
472. 1,1,4,4-Tetraethoxy-2-butyne	27	bp ²	97–102°	27	24
	...	<i>n</i> _D ²⁰	1.4346	27	...
473. Diketene	...	bp ²⁸	43°	38	24
474. Hydrocyanic acid	See Chap. 2	...		...	See Chap. 2
475. Sodium cyanide	Commercial	...		...	24
476. Triallyl orthoformate	...	...		...	24
477. 2-Propynyl formate	27	bp	105–109°	27	24, 33
	...	<i>n</i> _D ^{18.5}	1.4203	27	...
478. Allyl formate	...	...		...	24
479. α , β -Dichlorovinyl acetate	49c	bp ¹²	41–43°	49c	24
480. β -Triazoethyl acetate	21	bp ²⁰	74°	21	24
	...	<i>n</i> _D ²⁰	1.4345	21	...
	...	<i>d</i> ₄ ²⁴	1.123	21	...
481. Acetyl fluoride	12	bp	20–22°	12	24
482. Acetyl azide	21	...		...	...
483. Acetonitrile-boron trifluoride addition product	39	mp	118–120°	39	24
484. Methyl selenolacetate	22	bp ³²	29–31°	22	...
	...	bp	112–114°	22	...
485. Sodium chloroacetate	Commercial	...		...	24
486. Chloroacetyl fluoride	49n	bp ⁷⁵⁰	366.2	26	24
	...	vol ²⁰	74–76°	49n	...
487. Sodium bromoacetate	...	...		...	24
488. Bromoacetyl bromide	Commercial	...		...	24, 33
489. Sodium iodoacetate	...	...		...	24
490. Ethyl iodoacetate	40k	...		...	24, 33
491. Propiolic acid	27	bp ³⁵	73–77°	27	24, 33
492. Methyl propiolate	27	bp	100–102°	27	24, 33
493. Ethyl propiolate	27	bp	119–120°	27	24, 33
494. β -Chloroethyl propiolate	27	bp ¹⁷	79–82°	27	24, 33
	...	<i>n</i> _D ²⁰	1.4588	27	...
495. Allyl propiolate	27	bp ⁶⁰	70–73°	27	24
	...	<i>n</i> _D ^{18.5}	1.4378	27	...
496. Bromopropiolic acid	27	mp	85.5–87°	27	24, 33
497. Methyl bromopropiolate	27	bp ⁵	40–45°	27	24
	...	<i>n</i> _D ²⁵	1.4884	27	...
498. Acrylonitrile	Commercial	bp	75.5–76°	...	24
499. Methyl α -chloroacrylate	7	bp ⁵⁰	51–55°	7	...
	...	<i>n</i> _D ²⁰	1.4400	7	...
	...	<i>d</i> ₄ ²⁰	1.201	7	...
500. β -Chloroacrylonitrile	...	...		...	24
501. α,β -Dichloroacrylonitrile	Commercial	bp ⁶⁰	58–59°	...	24, 33
502. α,β,β -Trichloroacrylonitrile	48	mp	17–19°	48	24
	...	bp ⁷⁴⁰	141–142°	48	...
503. Ethyl β -chloropropioniminoester hydrochloride	12	mp	96°d	12	...
504. α,α,β -Trichloropropionitrile	...	...		...	24
505. Methoxytetrollic acid	27	bp ³	114–118°	27	24, 33
	...	<i>n</i> _D ²⁰	1.4669	27	...
506. Methyl methoxytetrolate	27	bp ³	56–58°	27	24, 33
	...	<i>n</i> _D ²⁰	1.4438	27	...
507. Crotonyl fluoride	49e	bp	88°	49e	24
508. Ethyl vinylacetiminoester hydrochloride	12	mp	90–100°d	12	...
509. Allyl cyanide	...	...		...	24
510. γ -Chlorocrotononitrile	49m	bp ¹⁰	60–62°	49m	24
511. β -Chlorocrotononitrile	48	bp ⁷³⁵	125.5–126.5°	48	24
512. Butyryl fluoride	49e	bp	65–67°	49e	24
513. Methyl α -chloroisobutyrate	...	...		...	24
514. α -Triazobutyric acid	21	bp ^{0.7}	80°	21	24, 33
	...	<i>n</i> _D ²⁵	1.4536	21	...
515. Methyl α -nitro- β -methylcrotonate	49p	bp ²⁴	120–125°	49p	...

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
516. Methyl methoxyacetate	...	...	...	24
517. Dimethyl diglycolate	Commercial	...	...	24
518. <i>bis</i> (β -Chloroethyl) diglycolate	7	bp ²	195–199°	7
519. Formaldehyde cyanohydrin	...	...	...	24
520. Methyl 2,2,2-trichlorolactate	49q	bp ⁸	92–94°	49q
521. Chloralcyanohydrin	49h	mp	59–60°	49h
522. β -Cyanoethyl nitrite	49d	cannot be distilled	49d	...
523. 1-Chloro-1-isonitrosoacetone	19	mp	108–109°	19
524. 1-Chloro-2-methylglyoxime	19	mp	183–184°d	19
525. Trichloroacetylcyanide	49g	bp	118–121°	49g
526. Vinyl mucochlorate	Commercial	vol ²⁰	0.289	26
527. Hexachlorodimethyl oxalate	49h	mp	79–80°	49h
528. Oxalyl fluoride	10	bp	approx. 2–3°	10
529. Oxalyl chloride	12	bp	64–65°	12
530. Methyl cyanoformate	42e	bp	98–99°	42e
531. Chlorocyanoformaldoxime	42d	bp ³	53–54°	42d
	...	mp	54–56°	42d
532. Cyanogen	...	...	...	24
533. Diethyl dichloromalonate	17	bp ¹⁴	115–116°	17
	...	n_D^{25}	1.4386	17
534. Diethyl ethoxymethylenemalonate	47c	n_D^{20}	1.4600–1.4620	47c
535. Ethoxymethylenemalononitrile	49j	mp	60–63°	49j
536. Diethyl diethoxymethylmalonate	47d	bp ^{7.5}	133°	47d
	...	n_D^{20}	1.4220	47d
537. Dimethyl acetylenedicarboxylate	49e	bp ²⁰	1.17	26
	...	vol ²⁰	98–99°	49e
538. Diethyl acetylenedicarboxylate	27	bp ³	84–88°	27
	...	n_D^{20}	1.4435	27
539. <i>bis</i> (β -Chloroethyl) acetylenedicarboxylate	27	bp ⁴	175–215°d	27
	...	$n_D^{18.5}$	1.5004	27
540. Diallyl acetylenedicarboxylate	27	bp ⁴	112–118°	27
	...	$n_D^{18.5}$	1.4718	27
541. Diisopropyl acetylenedicarboxylate	27	bp ⁴	103–107°	27
	...	$n_D^{18.5}$	1.4408	27
542. <i>bis</i> (2-Ethylhexyl) acetylenedicarboxylate	...	...	...	24
543. Dimethyl maleate	27	bp	199–204°	27
544. Dimethyl fumarate	...	bp	189–192°	27
545. Diallyl fumarate	27	bp ²⁰	137–140°	27
546. Fumaryl chloride	48	bp ¹³	62–63°	48
547. Dimethyl chloromaleate	...	...	...	24, 33
548. Diethyl bromomaleate	49g	bp ^{0.5}	85–86°	49g
549. Diethyl chlorofumarate	27	bp ²⁰	137–139°	27
550. Chlorofumaronitrile	Commercial	...	...	24, 33
551. Diethyl bromofumarate	27	bp ⁸	120–123°	27
552. Chlorofumaryl chloride	27	bp ²¹⁰	140–143°	27
553. Dimethyl dibromomaleate	17	bp ¹¹	134–136°	17
554. Diethyl α,α' -dichlorosuccinate	17	bp ³	106–108°	17
555. Dimethyl α,α' -dibromosuccinate	17	mp	60–61°	17
556. Dimethyl tetrachlorosuccinate	...	...	...	24
557. Dimethyl α,α' -dichloroglutarate	17	bp ¹	95–96°	17
558. Dimethyl α,α' -dichloroadipate	17	bp ²	126–128°	17
	...	n_D^{25}	1.4660	17
559. <i>bis</i> (Trichloromethyl) carbonate	...	...	...	24
560. Methyl β -chloroethyl carbonate	...	...	...	24, 33
561. <i>bis</i> (β -Chloroethyl) carbonate	12	bp ¹⁵	119–122°	12
562. Methyl fluorocarbonate	49g	bp	43–45°	49g
563. Methyl chlorocarbonate	...	...	...	24
564. Trichloromethyl chlorocarbonate (diphosgene)	See Chap. 3	...	...	See Chap. 3
565. Ethyl chlorocarbonate	...	...	...	24, 33
566. β -Chloroethyl chlorocarbonate	7	bp ⁷⁵²	152.5°	7
	...	n_D^{20}	1.4465	7
	...	d_4^{20}	1.3825	7

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
567. Allyl chlorocarbonate	49f	bp	107–111°	49f 24
568. Methyl triazoformate	49i	bp	97–101°	49i 24
569. Carbonyl chlorofluoride	See Chap. 3	...		See Chap. 3
570. Carbonyl chloride (phosgene)	See Chap. 3	...		See Chap. 3
571. N,N-Dichlorourethane	49d	...		24, 33
572. Dimethyl azoformate	7	bp ¹⁸	98°	7 24
	...	bp ⁴²	104°	7 ...
	...	n_D^{20}	1.4180	7 ...
	...	d_{20}^{20}	1.222	7 ...
573. <i>bis</i> (β -Chloroethyl) azoformate	7	bp ¹	140–143°	7 33
	...	n_D^{20}	1.4752	7 ...
	...	d_4^{28}	1.390	7 ...
574. Cyanogen chloride	See Chap. 2	...		See Chap. 2
575. Dichloroformoxime (phosgene oxime)	42a, 56c, 62, 64, 66	bp ²³	47–49°	42a 24, 55b
	...	mp	39–40°	63
	...	bp	129°	63 ...
576. Cyanogen bromide	Commercial	...		24
577. Dibromoformoxime	55b	...		55b
578. Methyl chlorothiolformate	42c	bp	111–112°	42c 24
	...	n_D^{20}	1.4901	42c ...
	...	d^{20}	1.290	42c ...
579. Trichloromethyl chlorothiolformate	42c	bp ²⁶	153–162°	42c 24
	...	n_D	>1.52	42c ...
	...	d^{20}	1.654	42c ...
580. Thiophosgene	42c	bp	73–76°	42c 24
581. Thiocarbonyl chloride polymer	42c	...		24
582. Acetyl thiocyanate	49h	bp ⁵¹	60.5–61.0°	49h 24
583. Carbomethoxy isothiocyanate	49h	bp ⁴⁸	58–61°	49h 24
584. Methyl thiocyanate	28	bp ⁷⁴⁰	128–129°	28 24
	...	n_D^{25}	1.4681	28 ...
	...	d_{31}^{24}	1.0732	28 ...
585. 2-Chloroethyl thiocyanate	5	...		24, 33
586. Hexyl thiocyanate	28	bp ^{1.5}	84–87°	28 24
	...	n_D	1.5650	28 ...
	...	d_{31}^{24}	0.941	28 ...
587. Dodecyl thiocyanate	28	bp ¹⁵	177–179°	28 24
	...	n_D^{33}	1.460	28 ...
	...	d_{25}^{25}	0.8958	28 ...
588. Ethylene dithiocyanate*	28	mp	90.5–91.5°	28 24
589. α,α -Dithiocyanopropane*	...	...		...
590. <i>bis</i> (Isothiocyanomethyl) ether*	...	...		24
591. Acetonyl thiocyanate	49e	...		24
592. Cyanogen sulfide*	...	...		...
593. Methyl chlorodithioformate	42c	bp ¹³	47–49°	42c 24
594. Allyl selenourea*	...	...		...
595. 1,3-Diselenocyanopropane*	...	...		...
596. Cyanogen diselenide*	...	...		...
597. β -Chloroetheneseleninyl chloride*	23	...		24
598. Ethaneseleninic acid	23	...		...
599. Ethaneseleninyl chloride hydrate	23	mp	72–75°	23 24
600. N,N-Dimethylformamide	Commercial	...		24
601. 2,5- <i>bis</i> (N-Methylcarbamyloxy)-3-hexyne (two forms)	...	...		35
602. N,N-Dimethylcarbamyyl fluoride	49k	bp ⁸³	65°	49k 24
603. Dimethylcarbamyyl chloride	11	bp	166–168°	11 24
604. Methyl isocyanate	37	bp	37–39°	37 35
605. Dimethylsulfamyl fluoride*	49n	bp ¹⁶	48.5°	49n 24, 55d
606. Dimethylsulfamyl chloride	11	bp ^{0.4}	34°	11 24, 33, 55d
607. N,N-Diethylchloroacetamide	Commercial	...		24, 33
608. N,N'-Diethyloxamide	11	mp	175°	11 24
609. Ethyl N,N- <i>bis</i> (β -chloroethyl) carbamate	39	bp ¹¹	131–132°	39 24
	...	n_D^{25}	1.4688	39 ...
	...	d_4^{25}	1.214	39 ...

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TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data	
		Property	Reference		
610. N-Butylmaleimide	...	...	...	24	
611. Dibutylcarbamyl chloride	11	bp ³	108–109°	11	24,33
612. 4-Chloropentyldiethylamine hydrochloride	34	mp	99.5–100°	34	24
613. Ethylenediamine thiosulfate	...	...	...	...	24
614. 1,6-Hexanediamine	Commercial	...	...	...	24
615. 1,6-Hexanediol diisocyanate	Commercial	...	...	...	24, 33
616. β -Dimethylaminoethyl formate	14	bp ⁷⁴⁵	125–130°	14	24, 33
	...	n_D^{20}	1.4262	14	...
	...	d_{20}^{20}	0.905	14	...
617. β -Dimethylaminoethyl acetate	14	bp ⁷⁴¹	148–151°	14	24, 33
	...	n_D^{20}	1.4178	14	...
	...	d_{20}^{20}	0.928	14	...
618. β -Methylethylaminoethyl formate	14	bp ⁷⁴¹	147–150°	14	24, 33
	...	n_D^{20}	1.4287	14	...
	...	d_{20}^{20}	0.919	14	...
619. β -Methylethylaminoethyl acetate	14	bp ⁷⁴¹	162–163°	14	24, 33
	...	n_D^{20}	1.4226	14	...
	...	d_{20}^{20}	0.918	14	...
620. Formylcholine chloride	14	mp	144–146°	14	24
621. Acetylcholine chloride	Commercial	...	...	...	24
622. Carbaminoylcholine chloride (Doryl)	...	...	...	...	35
623. β -(N-Methylcarbamyloxy)ethyltrimethylammonium chloride	49b	...	...	...	24, 33
624. β -(N-Propylcarbamyl)choline iodide	...	...	...	...	24
625. β -(N-Butylcarbamyl)choline iodide	...	...	...	...	24
626. β -Diethylaminoethyl formate	14	bp ⁷⁴⁵	157–160°	14	24, 33
	...	n_D^{20}	1.4358	14	...
	...	d_{20}^{20}	0.900	14	...
627. β -Diethylaminoethyl acetate	14	bp ⁵²	101–103°	14	24, 33
	...	n_D^{20}	1.4259	14	...
	...	d_{20}^{20}	0.911	14	...
628. β -Diethylaminoethyl carbamate ethiodide	29	mp	150–150.5°	29	24
629. β -Diethylaminoethyl N-methylcarbamate ethiodide	29	mp	90–92°	29	24
630. β -Dibutylaminoethyl carbamate butiodide	29	mp	99.5–100.5°	29	24
631. β -Dibutylaminoethyl N-methylcarbamate butiodide	29	mp	100–101.5°	29	24
632. <i>bis</i> (β -Hydroxyethyl)methylamine	Commercial	...	...	...	24
633. β -Methylhydroxyethylaminoethyl formate	14	bp ⁷	126–127°	14	24, 33
	...	n_D^{20}	1.4698	14	...
	...	d_{20}^{20}	1.045	14	...
634. Methyl- <i>bis</i> (β -formoxyethyl)amine	14	bp ⁷	110–111°	14	24, 33
	...	n_D^{20}	1.4501	14	...
	...	d_{20}^{20}	1.101	14	...
635. Methyl- <i>bis</i> (β -acetoxyethyl)amine	46b, 46c	bp ^{0.5}	59–60°	46b	33, 35
	...	n_D^{25}	1.439	46b	...
636. <i>bis</i> (β -Hydroxyethyl)ethylamine	Commercial	...	...	...	24
637. γ -Dibutylaminopropyl carbamate butiodide	29	mp	122.5–123.5°	29	24
638. γ -Dibutylaminopropyl N-methylcarbamate butiodide	29	mp	110.5–112°	29	24
639. γ -Diamylaminopropyl carbamate amyliodide	29	mp	108–110°	29	24
640. γ -Diamylaminopropyl N-methylcarbamate amyliodide	29	mp	78–83°	29	24
641. 1-Diethylamino-2,3- <i>bis</i> (N-methylcarbamyloxy) methiodide	29	mp	122–123.5°	29	24
642. Ethyl diazoacetate	21	bp ¹²	42–43°	21	24
	...	n_D^{18}	1.4592	21	...
643. Dimethylaminoacetonitrile	49h	bp ¹¹	55–56°	49h	24
644. <i>tris</i> (β -Thiocyanoethyl)amine	...	...	...	...	51
645. Trifluoromethylsilicane	...	...	...	...	24
646. Trichloromethylsilicane	...	...	...	...	24
647. Dichlorodimethylsilicane	...	...	...	...	24

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
648. Chlorotrimethylsilicane	...	...	...	24
649. Ethyltrifluorosilicane	...	...	...	24
650. Ethyltrichlorosilicane	40n	bp ⁷⁵⁰	96-98°	40n
651. Tetraethylsilicane	40f	...	...	24
652. Trifluoropropylsilicane	...	...	...	24, 33
653. Trichloropropylsilicane	...	...	...	24
654. Trichloroisopropylsilicane	...	...	...	24
655. Butyltrifluorosilicane	...	...	...	24
656. Butyltrichlorosilicane	...	...	...	24
657. Tributylboron	40a	...	...	...
658. Hexachlorocyclohexane (impure)	Commercial	...	...	24
659. α -Bromo-2-chloro-6-nitrotoluene	Commercial	...	...	24, 33
660. 2-Nitro-1-phenylpropene	18	bp ¹⁰	64.5-65.5°	18
	...	mp	139°	18
661. 1,2-bis(β -Chloroethyl)benzene	32	bp	120-122°	32
662. Phenyl chlorocarbonate	...	...	...	24, 33
663. 2,4,6-Trichlorophenyl chlorocarbonate	...	...	...	24, 33
664. Picryl silicate	40a	...	...	...
665. Cyclohexyl dithiocyanate*	...	...	...	...
666. <i>o</i> -Chlorophenyl thiocyanate*	...	...	...	...
667. <i>m</i> -Chlorophenyl thiocyanate*	...	...	...	...
668. <i>p</i> -Chlorophenyl thiocyanate*	...	...	...	...
669. <i>o</i> -Bromophenyl thiocyanate*	...	...	...	...
670. Phenyl selenocyanate*	...	...	...	...
671. <i>o</i> -Chlorophenyl selenocyanate*	...	...	...	...
672. <i>o</i> -Nitrophenyl selenocyanate*	...	...	...	...
673. <i>p</i> -Nitrophenyl selenocyanate*	...	...	...	...
674. <i>o</i> -Tolyl thiocyanate*	...	...	...	...
675. <i>o</i> -Tolyl selenocyanate*	...	...	...	...
676. <i>m</i> -Tolyl selenocyanate*	...	...	...	...
677. <i>o</i> -, <i>m</i> -, and <i>p</i> -Chlorobenzyl thiocyanates*	...	...	...	...
678. Benzyl selenocyanate*	...	...	...	...
679. <i>o</i> -Nitrobenzyl selenocyanate*	...	...	...	...
680. 2,4-Dinitrobenzyl selenocyanate	...	...	...	...
681. Benzylisothiuronium chloride	...	...	...	24
682. 3,5-Dimethyl-4-nitrosophenol	31	mp	179°d	31
683. Pyrocatechol sulfite	...	...	...	24
684. 3-Allylpyrocatechol	4	bp ¹	104-115°	4
	...	n_D^{25}	1.5660	4
	...	d_{25}^{25}	1.129	4
685. 4-Allylpyrocatechol	4	mp	44.5°	4
686. 3-(1-Vinylethyl-)pyrocatechol	4	bp ⁶	138-143°	4
	...	n_D^{25}	1.5536	4
	...	d_{25}^{25}	1.10	4
687. 3-Benzylpyrocatechol	4	bp ^{0.1-0.2}	110-160°	4
	...	$n_D^{26.5}$	1.5911	4
	...	$d_{26.5}^{26.5}$	1.141	4
688. 3-Cinnamylpyrocatechol	4	bp ^{0.1}	165-200°	4
	...	n_D^{27}	1.6045	4
	...	d_{27}^{27}	1.161	4
689. 3-Geranylpyrocatechol	4	bp ^{0.05-0.1}	150-200°	4
	...	n_D^{25}	1.5440	4
	...	d_{25}^{25}	1.031	4
690. 3-Tridecenylpyrocatechol	...	...	...	24
691. <i>p</i> -Anisyl selenocyanate*	...	...	...	...
692. <i>p</i> -Phenetyl selenocyanate*	...	...	...	...
693. <i>p</i> -Benzyloxyphenyl selenocyanate*	...	...	...	...
694. <i>p</i> -Nitrobenzyloxyphenyl selenocyanate*	...	...	...	...
695. <i>p</i> -Phenylene diselenocyanate*	...	...	...	...
696. Leuconic acid	36	...	...	24
697. <i>d</i> -Camphorimine nitrate	44d	mp	162-163°d	44d

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
698. N-Methyl- <i>d</i> -camphorimine	44d	bp ³	52–53°	44d
	...	bp ⁷⁵⁰	203–204°	44d
	...	<i>n</i> _D	1.4833	44d
	...	<i>d</i> ²⁵	0.9214	44d
699. <i>d</i> -Camphoroxime	44e	mp	119–120°	44e
	...	bp ²⁰	141°	44e
700. α -Chloroacetophenone	...	...		...
701. α,α -Dichloroacetophenone	...	...		...
702. α,o -Dichloroacetophenone	...	...		...
703. α -Chloro- <i>o</i> -nitrosoacetophenone	47e	mp	138°d	47e
704. α -Chloro- <i>p</i> -phenylacetophenone	...	...		...
705. Phenylpropargyl aldehyde	27	bp ¹⁷	114–117°	27
	...	<i>n</i> _D ²⁵	1.6029	27
706. Phenylpropargyl acetal	27	bp ¹⁹	153–156°	27
	...	<i>n</i> _D ²⁵	1.5160	27
707. α -Bromopropiophenone	...	...		...
708. Selenocyanoacetophenone*	...	...		...
709. 3-Isonitrosocamphor (d)	44e	mp	154–155°	44e
	...	bp ¹⁰	179°	44e
710. 1,3,5- <i>tris</i> (Chloroacetyl)benzene	32	mp	148–150°	32
711. 2-Methyl-1,4-naphthoquinone	...	...		...
712. <i>m</i> -Nitrobenzoylazide	21	mp	67–68°	21
713. α -Bromobenzoylcyanide	59	mp	29°	59
	...	bp ¹¹	132–134°	59
714. Methyl phenylpropiolate	27	bp ²	94–99°	27
	...	<i>n</i> _D ³⁰	1.5612	27
715. α -Amyl-N-(diethylaminoethyl)cinnamide hydrochloride	45	mp	102°	45
716. β -Benzoylpropionolactone	49d	mp	89–90°	49d
717. α -Chloro- <i>o</i> -cyanoacetophenone	47f	mp	118–119°	47f
718. N-Bromomethylphthalimide	8	mp	147–150°	8
719. Benzylidenemalononitrile	8	mp	82–83°	8
720. <i>o</i> -Chlorobenzylidenemalononitrile	8	mp	94–95°	8
721. <i>o</i> -Bromobenzylidenemalononitrile	8	mp	89.8–90.5°	8
722. <i>m</i> -Nitrobenzylidenemalononitrile	8	mp	104–105°	8
723. 3-Methylamino- <i>d</i> -borneol hydrochloride	43	...		...
724. β -(N-Phenylcarbamyl)choline iodide	...	...		...
725. Tetranilinosilicon	40h	...		...
726. Phenylimidophosgene	...	vol ²⁰	1.85	26
727. Hexanitrodiphenylamine	...	...		...
728. Trimethyl(2-phenylaminoethyl) ammonium chloride	...	...		...
729. 1,2,3,4-Tetrahydro-N,N-dimethyl-2-naphthylamine methochloride	41b	mp	221°	41b
730. 1,2,3,4-Tetrahydro-N,N-dimethyl-2-naphthylamine methiodide	41b	mp	222°	41b
731. 4-Dimethylamino-3-isopropylphenol methiodide	...	...		...
732. <i>m</i> -(Diethylamino)phenol methochloride	37	mp	180–182°d	37
733. 4,4'-Dithiocyanodiphenylamine	...	...		...
734. <i>p</i> -Selenocyanoaniline*	...	...		...
735. <i>p</i> -Selenocyanodimethylaniline*	...	...		...
736. N-Vanillylmandelamide	9	mp	105–106°	9
737. N-Vanillyl-10-hendecenamide	9	mp	60–61°	9
738. N-Methyl-N'-(4-hydroxy-2,3,5-trimethylphenyl)-urea	30	mp	226–227°	30
739. N,N'-Dicarbomethoxy- <i>p</i> -phenylenediamine	49g	mp	207–209°	49g
740. N,N'-Dicarbomethoxy- <i>p</i> -phenylenediamine	34	mp	192–193°	34
741. N,N'-Dicarbomethoxy-2,5-dichloro- <i>p</i> -phenylenediamine	41a	...		...
742. N,N'-Dicarbomethoxy-2,6-dichloro- <i>p</i> -phenylenediamine	34	mp	178–180°	34

TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
743. N,N'-bis(β -Chlorocarbethoxy)- <i>p</i> -phenylenediamine	34	mp	201°	34 24
744. 1,4-bis(N,N'-Dimethylureido)benzene	30	mp	229.5–230.5°	30 24
745. N,N'-Disulfinyl- <i>p</i> -phenylenediamine	46a	mp	115–116°	46a 35
746. <i>p</i> -Dimethylaminoaniline	Commercial	...		... 24, 33
747. <i>p</i> -Dimethylaminophenyl isothiocyanate	34	bp ⁵	148–150°	34 24
	...	mp	69–70°	34 ...
748. <i>p</i> -Dimethylaminophenyl isothiocyanate hydrochloride	34	mp	144–145°	34 24
749. <i>p</i> -Dimethylaminophenyl isothiocyanate methiodide	34	mp	171°	34 24
750. N-Methyl-N-(<i>p</i> -dimethylaminophenyl)thiourea hydrochloride	...	...		... 24
751. N,S-Dimethyl-N'-(<i>p</i> -dimethylaminophenyl)-thiourea hydroiodide	...	...		... 24
752. N,N'-Dimethyl- <i>p</i> -phenylenediamine	34	bp ¹⁷	157–160°	34 24, 33
	...	mp	53–54°	34 ...
753. N,N'-Dimethyl- <i>p</i> -phenylenediamine dihydrochloride	34	mp	224°d	34 24
754. N,N'-Dicarbethoxy-N,N'-dimethyl- <i>p</i> -phenylenediamine	34	mp	106–107°	34 24
755. N,N,N',N'-Tetramethyl- <i>o</i> -phenylenediamine	34	bp ¹²	92–93°	34 24, 33
756. N,N,N',N'-Tetramethyl- <i>o</i> -phenylenediamine methiodide	34	mp	194°d	34 24
757. N,N,N',N'-Tetramethyl- <i>m</i> -phenylenediamine	34	bp ¹⁰	121–124°	34 24, 33
758. N,N,N',N'-Tetramethyl- <i>m</i> -phenylenediamine methiodide	34	mp	187°d	34 24
759. N,N,N',N'-Tetramethyl- <i>p</i> -phenylenediamine	Commercial	...		... 24, 33
760. N,N,N',N'-Tetramethyl- <i>p</i> -phenylenediamine dihydrochloride	34	mp	222°d	34 24
761. N,N,N',N'-Tetramethyl- <i>p</i> -phenylenediamine methiodide	34	mp	266°d	34 24
762. <i>p</i> -Phenylene-bis(oxazolidone-3)	34	mp	253–254°	34 24
763. N',N'-Diethyl-N,N-dimethyl- <i>p</i> -phenylenediamine	34	bp ¹⁰	137°	34 24, 33
	...	mp	263–265°	34 ...
764. N,N'-Diethyl-N,N'-dimethyl- <i>p</i> -phenylenediamine	34	bp ¹⁷	150–151°	34 24, 33
765. N,N,N'-Triethyl-N'-methyl- <i>p</i> -phenylenediamine	34	bp ¹⁰	144°	34 24, 33
	...	mp	22°	34 ...
766. N,N,N'-Triethyl-N'-methyl- <i>p</i> -phenylenediamine dihydrochloride	34	mp	220°d	34 24
767. N,N,N'-Triethyl-N'-methyl- <i>p</i> -phenylenediamine methiodide	34	mp	177°	34 24
768. N,N,N',N'-Tetraethyl- <i>p</i> -phenylenediamine	34	bp ¹⁴	155–156°	34 24, 33
	...	mp	51–52°	34 ...
769. N,N,N',N'-Tetraethyl- <i>p</i> -phenylenediamine methiodide	34	mp	185°	34 24
770. N,N'-bis(β -Hydroxyethyl)- <i>p</i> -phenylenediamine	34	mp	123°	34 24
771. N,N'-bis(1-Methyl-4-diethylaminobutyl)- <i>p</i> -phenylenediamine	34	bp ¹⁰⁻⁶	145°	34 24
772. N-Methyl-N'-(<i>p</i> -dimethylaminomethylphenyl)-thiourea hydrochloride	...	...		... 24
773. Polymer of N,N'-decamethylene-N,N'-dimethyl-4,4'-diaminodiphenylmethane bis-methobromide	...	...		... 24
774. Phenylhydrazine	Commercial	...		... 24
775. N-Carbomethoxy-N'-phenylhydrazine	49f	mp	114.5–116°	49f 24
776. <i>p</i> -Phenylenedihydrazine dihydrochloride	34	dec.p.	200°	34 24
777. Tetra- <i>m</i> -nitrophenylsilicon	40a	...		... 24
778. Tetrahydrofurfuryl alcohol	Commercial	...		... 24
779. Tetrahydrofurfuryl fluorocarbonate	49r	bp ³⁵	92–94°	49r 24
780. Tetrahydrofurfuryl chlorocarbonate	49o	bp ¹⁰	81–83°	49o ...

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TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
781. 3,6-Epoxy cyclohexene	...	...	...	24, 33
782. Adduct of furan and maleic anhydride	7	mp	110–111°	24, 33
783. Furan	Commercial	...	...	24
784. Methylfuran	Commercial	...	...	24
785. 1-(2-Furyl)-2-nitroethylene	18	bp ¹⁰	74–75°	24, 33
786. 1-(2-Furyl)-2-nitropropene	18	mp	110°	...
		bp ¹⁰	48.5–49.5°	24, 33
		mp	125°	...
787. Furfuryl alcohol	Commercial	...	...	24
788. 2-Furaldehyde	Commercial	...	...	24
789. Furoic acid	Commercial	...	...	24
790. 5-Hydroxy-2-chloromethyl- γ -pyrone	12	mp	162–163°	...
791. 5-Methoxy-2-chloromethyl- γ -pyrone	12	mp	119–120°	24
792. 5-Hydroxy-2-dimethylaminomethyl- γ -pyrone methochloride	49p	mp	245°d	24
793. Tetrafurylsilicon	...	...	...	24
794. Isobutylenimine	32	bp	69–70°	24
795. N-Isopropylethylenimine	32	bp	65.5–66.5°	24
796. N-Phenylethylenimine	32	...	...	24
797. Azetidine	...	...	...	24
798. Heliotridene†	25	bp	164–166°	24
		n_D^{19}	1.4870	...
		bp ³⁰	111–112°	24
799. Chlororetronecane†	25	n_D^{19}	1.4913	...
		mp	122–123°	24
		mp	93–95°	24
802. Desoxyretronecine hydrochloride†	25	mp	181–183°	24
803. Platynecine†	25	mp	147–148°	24
804. Retronecine†	25	mp	119–121°	24
805. Monocrotaline†	25	mp	199–201°d	24
806. Diacetylretronecine†	25	bp ^{0.1}	101–108°	24
807. Diacetylretronecine methiodide†	25	mp	122–123°	24
808. 2-Methyloctahydropyrrocoline	25	bp ²⁶	70–72°	24, 33
		n_D^{18}	1.4667	...
		mp	226–227°	24
810. 4-Allyloctahydro-2-methylpyrrocolinium bromide	25	mp	258–259°d	24
811. Octahydro-2-methyl-4-(β -phenylethyl)-pyrrocolinium bromide	25	...	...	24
812. Octahydro-4-(β -hydroxyethyl)-2-methylpyrrocolinium bromide	25	...	...	24
813. 4-(β -Acetoxyethyl)-octahydro-2-methylpyrrocolinium chloride	25	...	...	24
814. 2-Triacetylnorcholyloctahydropyrrocoline	25	mp	75–95°	24
815. N-Chlorocarbamylpiperidine	...	...	...	24
816. β -(Piperidyl-N-carbamyl)choline iodide	...	...	...	24
817. 1-Piperidylsulfamyl chloride	...	...	...	24
818. N- β -Hydroxyethylpiperidine	12	bp ²⁰	95–96°	...
819. 2-Piperidylloethyl N-methylcarbamate methiodide	29	mp	103–105°	24
		bp ¹¹	83–84°	24
		...	...	...
822. Conine (α -propylpiperidine)	Commercial	...	...	24
823. 2-(β -Hydroxyethyl)pyridine	12	bp ^{0.2}	84–90°	...
824. 3-Bromoacetylpyridine hydrobromide	...	...	...	24
825. Nicotine	Commercial	...	...	24
826. 2-(N-Carbomethoxyamino)pyridine	49j	mp	122°	24
827. 4-(β -Dimethylaminoethyl)pyridine	30	bp ²⁰	135–145°	35
828. 4-(β -Dimethylaminoethyl)pyridine dimethiodide	30	mp	207–208°	35
829. 2-(β -Hydroxyethylamino)pyridine	12	bp ¹⁷	180–185°	...
		mp	109–110°	...

† These substances were obtained from natural sources.

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TABLE 1 (Continued)

Compound	Reference to synthesis	Physical properties		Refer. to toxicity data
		Property	Reference	
830. 2-Carbethoxyoxy-4-carbethoxyaminopyrindane	34	mp	139–141°	24
831. 4-Carbethoxyamino-2- <i>p</i> -tosyloxy-4-pyrindane	34	mp	132.5°	24
832. 4-Amino-2-hydroxypyridine	...	mp	309°	24
833. 4-Acetylamino-2-hydroxypyridine	...	...		24
834. 2-Acetoxy-4-acetylaminopyridine	...	...		24
835. 2-Methylpyrrocoline hydrochloride	25	mp	61–62°	24
836. 2-Phenylpyrrocoline	25	mp	214–215°	24
837. 3-Acetyl-2-methylpyrrocoline	25	mp	83–85°	24
838. 2-Triacetylnorcholylpyrrocoline	25	mp	169–170.5°	24
839. 1,2,3,4-Tetracarbomethoxyquinolizine	25	mp	186–188°	24
840. 4,7-Dichloroquinoline	47c	mp	84–85°	24
841. 2- <i>p</i> -Nitrophenylquinoline	40l	...		...
842. 8-Methoxy-5-methylquinoline	...	...		24
843. 2-(<i>m</i> -Dimethylaminophenyl)quinoline	40k	...		...
844. 2-(<i>p</i> -Dimethylaminophenyl)quinoline	...	...		24, 33
845. 2-(<i>p</i> -Dimethylaminophenyl)-3-bromoquinoline	40j	...		24
846. N-2-Naphthyl-1,2,3,4-tetrahydro-1,3-isoquinoline- linedione	Commercial	...		24
847. 9-Vinylcarbazole	...	...		24, 33
848. Ethylene-N-nitrosourea	19	mp	102–104°d	24
849. 1,4-Diethyl-1,4- <i>bis</i> (β-hydroxyethyl)piperazinium dichloride	...	...		24
850. 2-Phenylimidazo-[1,2- <i>a</i>] pyridine hydrobromide	25	mp	122–124°	24
851. N-Morpholinoacetonitrile	49i	mp	60°	...
852. 1,4-Selenoxan-4-dichloride*	...	...		...
853. Phenoxtellurine*	...	...		...
854. 10,10-Dichlorophenoxtellurine*	...	...		...
855. Bisapomethylbrucine hydrochloride	...	...		24
856. Bisapomethylbrucine diacetate	...	...		24
857. Dimethylfurazane	12	bp ⁷⁶⁰	153°	24
858. Dimethylfurazane oxide	12	bp ²⁷	170–171°	...
859. Dicarbethoxyfurazane oxide	12	bp ²⁶	173°	24
860. Methyl N-(5-tetrazalyl)carbamate	49g	mp	>300°	...
861. Product of thermal destruction of cyanogen chloride	...	...		35
862. Veratrine	Commercial	...		24
863. Ricin*	See Chap. 12	...		See Chap. 12
864. Ficin	...	...		24
865. Lubricating oil, S.A.E. No. 10	...	...		24
866. Fog oil, SGF No. 1	...	...		24

PART II

SPECIAL PHYSIOLOGICAL AND TOXICOLOGICAL STUDIES

SECRET

Chapter 15

THE ASSESSMENT OF PARTICULATES AS CHEMICAL WARFARE AGENTS

By William L. Doyle and R. Keith Cannan

15.1

INTRODUCTION

DURING THE YEARS 1941–1945, more than 1,500 compounds were examined in the United States as potential chemical warfare agents. The volatilities of the majority were so low that they could have little offensive value if used in the form of vapors.^{5,11} On the other hand, a few were intrinsically so much more toxic or more vesicant than were the standard chemical warfare agents^{6,10,12} that the question of dispersing them in particulate form commanded consideration. Ricin (W), for example, was several score times as toxic as phosgene, whereas 1,2-bis(β -chloroethylthio)ethane (Q), when applied in a solvent to the skin, was vesicant at one-tenth of the minimal blistering dose of mustard (H).

Apart from observations incidental to the study of the screening power of smokes, little attention was paid to the toxicological properties of particulate dispersions until the decision was made to submit finely powdered ricin to field tests. As a result of this decision, an expanding program of work was undertaken on the physical and toxicological assessment of dispersions of this material. As a result of the experience gained, the investigations were later extended to a study of the vesicant effects of aerosols of bis(β -chloroethylthioethyl) ether (T), Q, and tris(β -chloroethyl)amine (HN3).

The point of departure of all the work was an appreciation of the paramount importance of particle size in determining the effectiveness of a particulate cloud (see Table 1). In the first place, the particle size determines the stability of the cloud under given meteorological conditions. Secondly, it controls the fraction of the area dose which will impact upon an obstacle in the path of the cloud, and therefore determines the hazard to the unprotected skin and eyes of an individual in the cloud. Finally, the impacting characteristics of the particles also control the inhalation toxicity of the cloud, since they affect the fraction of the inhaled material that will penetrate to and be retained in the lungs.

The problem of the assessment of particle size in fine liquid particulates had been greatly advanced by

TABLE 1. Relation of particle diameter to chemical warfare characteristics.

Particle diameter (microns)	Type of cloud	Characteristics of clouds
$10^{-3} - 10^{-2}$	Vapor (molecular)	Airborne, nonpersistent, subject to laws of diffusion. Invades lungs, eyes, clothing, and skin.
10^{-1}	Aerosol	Airborne and nonpersistent. Invades lungs. Does not impact out of streamlines.
1 to 5	Fine particulates	As for aerosol except that it is more readily filtered and the lung retention is more complete. Five μ is close to upper limit of nasal penetration.
20 to 100	Particulates	Cloud persists in mild lapse conditions. Does not reach lungs. Impacts on surfaces and should invade eyes and skin.
200	Sprays	Sediment rapidly. Impact efficiently. Not dealt with in this chapter.

the British in the invention of the cascade impactor.³³ The attempt was made to adapt this instrument to the assessment of clouds of solid particles. However, the irregular size, shape, and density of the particles raised a number of difficulties which have not yet been satisfactorily resolved. Much fundamental work has, however, been carried out on the calibration and use of the cascade impactor with dusts.^{13,18}

The relation of particle size to the inhalation toxicity of toxic particulates was investigated by direct assays in animals of various species. Ricin aerosols of controlled ranges of particle size were utilized.¹⁸ⁱ At the same time the filtering characteristics of the human nose were measured by observations of the extent of penetration of a variety of nontoxic particulates.^{13d,18g,h} The effectiveness of dispersions of nonvolatile and of slightly volatile vesicants on human skin and on the eyes of animals were investigated as a function of particle size, wind speed, etc.^{13k}

The results obtained in this work have shown clearly that the significance of the size of the airborne particles cannot be reduced to any simple formula. However, the following broad conclusions would appear to be justified and may serve to indicate the status of the problem.

1. The size, shape, and density of the particles, as well as the wind speed and other meteorological conditions, all contribute to the aerial behavior of a particulate cloud. Particles with effective diameters greater than about $100\ \mu$ sediment rapidly in a stable atmosphere. They will remain airborne for significant periods only under conditions of considerable turbulence. On the other hand, clouds containing effective concentrations of particles smaller than $0.1\ \mu$ in diameter are subject to rapid aggregation. For example, if a cloud with a concentration of $1\ \text{mg/l}$ were composed initially of very small particles, it would attain relative size stability only when the average particle diameter had grown to about $0.7\ \mu$.

These considerations lead to the conclusion that the problem of toxicological effectiveness may be restricted to a consideration of clouds whose particles (if of unit density) fall within the size range of 0.1 to $100\ \mu$.

2. In general, toxic agents are much more effective if they enter the lungs than if they are retained in the nose. The probability that a particle will penetrate the nasal barrier increases as the size of the particle diminishes. Available evidence indicates that the optimum size for penetration to and retention in human lungs probably lies within the limits of 0.5 – $3\ \mu$ in diameter.^{18g,h} The optimum size for laboratory animals is appreciably lower.¹⁸ⁱ These values are for resting animals and are further lowered at the high ventilation rates associated with exercise.

3. The probability of the impaction of a particle on a surface in the path of a cloud increases with the size of the particle. At moderate wind speeds, the fraction of the area dose which may be expected to impinge on the surface becomes significant if the particle size is above $10\ \mu$ and becomes an important fraction of the area dose above a size of about $70\ \mu$.^{46,47}

It would appear from items 2 and 3 that no single dispersion can exploit to the full the potentialities of an agent which, like Q, is both vesicant by contact and toxic by inhalation. This is a fundamental dilemma which imposes serious limitations on the offensive potentialities of aerosols of this type.

4. If munitions were available which would disperse particulate material in either of the optimum

size ranges indicated above, new orders of inhalation and of vesicant effectiveness in the field should be obtainable. Such munitions have not yet been adequately developed.³⁹

15.2 TYPES OF PARTICULATES

15.2.1 Sternutators

Classical sternutators such as diphenylamine-chlorarsine (adamsite), diphenylaminecyanoarsine (cyan DA), and toxic sternutators such as aconitine and nitrophenyldichlorarsine are primarily harassing agents. These agents act at concentration time products (*Ct*'s) considerably below $0.1\ \text{mg min/m}^3$. At present there is little interest in these agents because they are stopped by available masks and because trained troops carry on effectively despite their presence. It is possible, however, that the utility of sternutators has not been adequately considered. Larger particles than those that have been utilized may be more harassing. German interest in mixtures of sternutators with mustard¹⁶ may indicate attempts to hide the presence of more toxic agents.

15.2.2 Toxic Particulates

1. Inorganic substances, e.g., cadmium selenium.
2. Synthetic organic compounds, e.g., aromatic carbamates.

3. Naturally occurring substances, e.g., ricin (W).
The metals are thermally stable and may be incorporated in standard smoke incendiary³⁸ or high-explosive weapons. As indicated in Chapter 11, these substances are not more toxic than standard chemical warfare agents, but they may be used without ready detection in various types of munitions.

Although the aromatic carbamates are considerably more toxic than standard agents,¹⁰ they are unstable in aqueous solution and to heat. For these reasons, little serious consideration has been given to their use as particulate clouds.

Ricin (W) is intrinsically somewhat more toxic than the best of the carbamates. It is also thermolabile. Its toxicity when dispersed as a cloud has been studied extensively in the laboratory and preliminary field trials, using special munitions, have been carried out. This interest in ricin was not entirely dependent on its own merits as a toxic agent. It was recognized as a prototype of toxic protein materials of bacterial origin which were known to have even greater toxicity but which were less conveniently prepared and handled.

15.2.3 Vesicants

1. Volatile vesicants, e.g., mustard (H), *tris*(β -chloroethyl)amine (HN3).

2. Nonvolatile vesicants, e.g.,^{1,2} *bis*(β -chloroethylthio)ethane (Q).

All the members of this group are toxic, but not so toxic as those in Section 15.2.2, (1) and (2). They are, however, vesicant. The best nonvolatile vesicants are intrinsically more toxic and more vesicant than the volatile ones. Q is inherently 10 to 20 times as vesicant as H and at least 5 times as toxic.^{7,9,13a,18b} They should be more difficult to detect than the volatile agents. In the field they will not be expected to create a vapor hazard, but, by contamination of equipment, should establish a contact hazard for bare skin which it would be difficult to eliminate by decontamination. It is doubtful if nonvolatile vesicants can be effective through clothing.^{13j,18b} The volatile members of this group can be dispersed thermally, by means of airplane spray, or by high explosive-chemical shells. The particle size achieved will markedly influence the action of the agent. Thus, very small particles (0.2–1.0 μ in diameter) may be nonvesicant because of streamlining, but will be more toxic by inhalation and will also yield the greatest and most rapid vapor return. Larger particles (5 to 25 μ) will have greater vesicancy, but probably at the expense of toxicity. The largest particles (200–2,000 μ) may be most effective for the penetration of clothing, particularly of the permeable protective type. The largest particles may also create a contact and traversal hazard.

These statements are broad and tentative generalizations based upon contemporary views of the characteristic behavior of particles of different diameter. Many of these generalizations require further experimental investigation. The results of contemporary work are reviewed in Section 15.5.1.

15.3 THE EFFECTIVENESS OF PARTICULATE CLOUDS

15.3.1 Stability

Sedimentation. The rate of sedimentation of particles in a static atmosphere increases with increase in particle size. Computations based upon kinetic considerations indicate that precipitation becomes rather rapid when the effective diameters of the particles exceed about 100 μ . Clouds of such large particles could be maintained in the air for significant

periods only under strongly turbulent conditions. They are sprays rather than clouds and find their natural use for contact and ground contamination — e.g., as airplane sprays. The optimum particle size for such sprays depends on a variety of factors — the speed of the plane, the turbulence, the volatility of the agent, etc. — which it is not the province of this chapter to discuss.¹⁹ Suffice it to say that for direct assault upon exposed personnel there is general agreement that the optimum range of particle size to obtain massive and diffuse contamination with a vesicant is about 0.3–2 mm in diameter. If ground contamination is the objective, the upper limit of size may be unimportant.

Coagulation. Although the rate of sedimentation sets the upper limit of size in a persistent aerosol, the tendency of particles to coalesce upon collision establishes a lower limit of particle size stability. On simple considerations of collision frequency, the half life of a particle should be roughly proportional to the concentration of the aerosol. With increase in particle size, the concentration required to give a fixed half life increases with the mass, and therefore with the cube of the radius of the particles. It has been estimated⁴⁷ that the half life of an aerosol containing 5×10^6 particles per milliliter is 6 minutes at room temperature. For particles of 0.1 μ in diameter and unit density, this corresponds with a concentration of 2.5 $\mu\text{g/l}$. For particles of 1 μ in diameter, the corresponding concentration is 2.5 $\mu\text{g/l}$. If these clouds were initially established in higher concentrations, they would aggregate until the numbers of particles in unit volume had fallen to a relatively stable level. It should be noted that the tendency to coagulate does not lead directly to a reduction in mass concentration, but rather to an increase in average particle size. The phenomenon is important, therefore, only if clouds of small particle size are required. There is no purpose in attempting to disperse a particulate in a smaller particle size than can be sustained by the concentration that is to be established. If a concentration of 1 $\mu\text{g/l}$ is accepted as the lowest which the toxicity of the material would justify, then the smallest particle size which it is worth while attempting to disperse is of the order of 1.0 μ .

In summary, therefore, considerations of rates of sedimentation and of coagulation suggest that we should concern ourselves with the behavior of airborne particles in the size range of 0.1–100 μ in diameter, corresponding to a 10^9 -fold range of particle mass.

15.3.2 The Significance of Particle Size

The tactical use of a chemical warfare agent in the form of a cloud is, in general, to be justified only when the conditions of the operation will be such that personnel exposed to the cloud will absorb casualty-producing doses through the lungs or through the skin. When the cloud is a true vapor, the actual dose that is inhaled under standard conditions of respiration can be predicted from the product of the concentration and the time of exposure (Ct). The amount of vapor absorbed from the skin under standard conditions of temperature and humidity can, likewise, be predicted from the Ct , since the rate of diffusion of the vapor to, and the rate of penetration of, the skin may generally be taken to be proportional to the concentration. It follows that an effective dosage on the target can be assured if munitions expenditure is properly adjusted to the meteorological conditions.

The tactical requirements cannot be formulated so simply when the cloud is composed of particles with colloidal or larger dimensions. In this situation, the concentration of the agent may actually be less important than the sizes of the airborne particles. It has been noted that this is a factor in determining the stability of a particulate cloud. In the case of an agent which is toxic by inhalation the particle size also controls the proportion of the inhaled material which is filtered out of the inspired air in the respiratory passages. Likewise, in the case of a vesicant, the amount of material deposited upon an exposed surface at a given Ct and wind speed is a function of the particle size. In brief, the effective dose of an inhalant and of a vesicant depends on the impinging characteristics of the particles which, in turn, depend upon the size and density of the particles.

15.3.3 The Impingement of Particles

The amount of an airborne particulate which will deposit on an object in the path of a cloud will be the sum of the amount which impinges upon the object and the amount which is deposited under gravity.⁴⁵ Since this discussion has been limited to clouds in which the rate of sedimentation is small, consideration may be confined to the amount which impinges on the object.

The probability that a spherical particle will impinge upon a cylindrical surface in its path is given⁴⁵ by

$$P = a \left(1 + \frac{d}{D} \right) (1 - e^{-\alpha u \rho c^2 / D}) \quad (1)$$

In this equation, u is the velocity of the particle, d its diameter, and ρ its density. D is the diameter of the target, while a and α are constants. It will be seen that the tendency to impinge increases with the size and with the density of the particle and also with the wind speed. It depends also on the size and shape of the target. According to Sell,⁴⁶ when $D \gg d$, $a = 0.75$ and $\alpha = 650$ cgs units. More recent experimental data give values of $a = 0.75$ to 1 and $\alpha = 70$ cgs units.

It may be assumed that all liquid particles which impinge upon a surface will remain adherent to it, but it is not expected that this will be true for a solid particulate. In this case the probability of adherence to the surface may be much lower than the probability of impingement. The magnitude of the losses will depend upon the nature of the surfaces of the target and of the particulate.

15.3.4 The Effective Vesicant Dose

The toxicologically effective dose of a nonvolatile vesicant may be taken to be proportional to the amount of the agent which is deposited on unit area of a surface exposed to the cloud in question. This amount is given by the product of Ct , u , and f , where u is the wind speed and f is an impactation factor. The latter corresponds with the fraction of the area dose which is deposited on the target. When the cloud is homogeneous with respect to size and density and the adhesiveness of the surface for the particles is high, f is given by P in equation (1). When the cloud is heterogeneous, it is necessary, in principle, to measure the distribution of the total concentration over the particle sizes which are present and to derive an overall impactation factor for the cloud.

P is an exponential function of the size of the particle. When its value is considerably less than unity, it increases rapidly with small increases in particle size. For this reason, the impacted dose of a heterogeneous cloud may be largely determined by the relatively small number of the larger particles which are present. A heterogeneous cloud may, therefore, be a much more effective vesicant than a homogeneous cloud having the same mass median diameter.

Further complications are introduced if, as may occur with solid particulates, the particles vary in shape and density as well as in volume. A very important example of such variations is the formation

of loose irregular aggregates of low density from smaller primary particles of uniform density. This type of aggregation is prone to occur during the dispersion of powdered materials, particularly if they are somewhat hygroscopic. The effects of these complicating factors on the impingement of solid particles are elaborated in Section 15.4.3.

When one is dealing with a particulate cloud of a slightly volatile agent such as HN3, consideration must be given to the toxic effectiveness of the vapor as well as to that of the dispersed phase. It must be remembered, also, that the characteristics of the cloud continually change with time. The particulate phase suffers progressive loss in concentration and size as volatilization proceeds until a pure vapor cloud results. An analysis has been made of the factors which determine the rate of evaporation of airborne particles.³⁷

It is of interest to note that the wind speed has two opposed effects on the tactical efficiency of a vesicant particulate cloud. The greater the velocity of the wind, the lower is the concentration of an agent which is being generated at a fixed rate. On the other hand, the greater the wind speed, the greater is the impaction efficiency of a given concentration of the particles.

Experimental studies of the relation of particle size to the vesicancy of aerosols of Q, T, and HN3 are reviewed in Section 15.5.1.

15.3.5 Effective Inhaled Dose

It is generally acknowledged that toxic particles are less effectively absorbed from the nasal and respiratory passages than from the alveoli of the lungs. Considering the pulmonary toxicity alone, the effective dose of an inhalant may be given as the product of Ct , v , and $(1-f)$ where v is the minute volume of respiration, and f is the fraction of the inhaled material which is trapped in the respiratory passages. It will be agreed that this fraction is determined in large measure by the amount of impaction in the nose. It may be expected to vary with the species of animal, and, to some extent, from animal to animal of the same species. It will also vary with the physiological state of a single animal. To the extent that impingement in the nose determines f , an increase in rate of respiration will, by increasing the velocity of the particles in the nasal passages, result in a greater nasal retention and a reduced effective dose.

The question of the extent to which particulate material which enters the alveoli is retained and ab-

sorbed has been investigated in a preliminary way. The results are summarized in Section 15.5.2.

Experimental studies of the effects of particle size on the toxicity of ricin for animals and on the retention of nontoxic particulates in the human nose are reviewed in Section 15.5 and in Chapter 12.

15.4 LABORATORY PRODUCTION AND CONTROL OF PARTICULATE CLOUDS

15.4.1 Dispersal

Liquids and Solutions. In a few special studies the Sinclair-LaMer homogeneous smoke generator has been used.^{1,3} Thermogenerators may be employed for the dispersal of stable, slightly volatile agents. In most cases, however, various types of atomizer have been used under conditions of operation which have been empirically determined to give clouds of the desired characteristics. Preliminary studies of the fundamental properties of atomizers have been reported.^{14b}

A useful method of producing clouds of varying particle size from a standard atomizer has been the following. A nonvolatile cosolvent is mixed in varying proportions with a dilute solution of the agent in a volatile solvent. When these mixtures are atomized, the mass median diameters of the particles in the cloud vary with the proportion of nonvolatile solvent in the original mixture. For example, glycerol has been found to be a satisfactory cosolvent for aqueous solutions of ricin and dibutyl phthalate for solutions of nonvolatile vesicants.^{13k,18b}

Solids. Electric arcs employing the toxic agent as one component of the electrodes provide useful sources of finely divided metals and their oxides. Thermal generation of toxic clouds by the incorporation of the agent in incendiary or fuel block mixes may also be used when the agent is thermostable. Such thermal generators tend, however, to give dispersions which coagulate rapidly.^{38,40,41}

The obvious alternatives in the case of a thermolabile solid such as ricin are to disperse by atomization of a solution or to generate a dust cloud from a finely comminuted powder. Most devices which have been described for the dispersal of powders lead to a fractionation of the sample. In some it is the smaller particles, in others, the larger particles which tend to disperse the more rapidly. In most there occurs a considerable formation of loose aggregates in the cloud. Although some attempts have been fairly successful,^{13b,18h} no really satisfactory method for the

uniform dispersal of a powder at a rate of a few milligrams a minute has been described. The devices which lead to least aggregation in the cloud have the disadvantage of a variable rate of delivery. (See Section 15.6 for dispersal in the field.)

15.4.2 Measurement of Size

The assessment of particle size in a cloud requires not only the observation of the range of sizes in the cloud, but also the amounts of material in the different size categories. The results are comprehensively expressed as curves in which the cumulative amount of material is shown as a function of the diameter of the particle. Three types of curves may be distinguished, according to whether the particle diameter is plotted against (1) the number, (2) the volume, or (3) the mass of airborne particles. From these curves may be derived respectively a number median diameter [NMD], a volume median diameter [VMD], and a mass median diameter [MMD]. The number distribution is appropriate if one is interested in effects dependent on the number rather than on the mass of airborne particles — as, for example, in the knockdown of mosquitoes. The volume distribution has no particular practical significance, but is the form in which results must be cast if the densities of the particles are not known and the amount of material must be evaluated from microscopic observations of the numbers and diameters of the particles in the sample. The mass distribution is the description of particle size which is most significant to the problem of the vesicant and toxic effects of the cloud.

The clouds from atomized liquids have fairly typical distributions and the densities of the particles are uniform. In such conditions the MMD is sufficient to characterize the cloud satisfactorily. In dust clouds generated from powders, on the other hand, the distribution of sizes may be quite abnormal, the unitary particles may be far from spherical, and many aggregates of low density may be present. The MMD of such a cloud may be a quite misleading index of the impaction efficiency of the cloud. The complete mass distribution is required for the characterization of such a cloud.

Methods. When dealing with dusts it is desirable to make counts of the undispersed material for comparison with the airborne cloud. The MMD and the range of sizes in a given preparation are best determined by direct microscopic examination if the MMD is below $10\ \mu$. The work is tedious and various methods have been discussed to save labor but critical in-

vestigators agree with Fairs⁴³ on the procedure to be followed. Hard and fast rules for the number of particles to be counted cannot be stated.^{13c} The statistical features of the problem are well presented by Dallavalle.⁴² The suitability of a laboratory or field procedure for the measurement of the particle size in a cloud depends upon the size of the particles, whether they are liquid or solid, upon the time available for sampling, and upon the concentration. Optical methods suited to the analysis of homogeneous smokes have been developed.² These methods are not readily applicable to heterogeneous clouds, but some attempts in this direction have been made.¹⁵ In general, the optical methods result in neglect of the relatively small numbers of coarse particles which may carry an appreciable fraction of the mass. An instrument capable of photoelectric measurement of the surface area of individual particles is not theoretically impossible. In view of the labor required in available procedures, some such device is highly desirable.

Ultramicroscopic and dark field observations of falling particles have frequently been employed.⁴⁸ Such methods are limited to particles small enough to remain airborne prior to observation and to concentrations so low that coagulation is avoided. There is great danger that large particles will be lost in the sampling procedures prior to observation.

The thermal precipitator⁴⁷ is very useful for particles below 5 to $10\ \mu$ in diameter, provided that the cloud is available for a sufficient period of time so that the necessarily slow sampling rate provides an adequate sample.

For clouds ranging from 2.0 to $50\ \mu$ in diameter, there is one instrument at present which avoids many of the difficulties inherent in other methods. This is the cascade impactor.^{26,33} It merits more detailed consideration than those already referred to.

15.4.3 The Cascade Impactor

This instrument consists of a series of four jets arranged in series so that the sampled cloud impinges at four increasing velocities on to suitably prepared microscope slides (A,B,C, and D). In this way the particles are separated into four impacted groups. The size ranges trapped on successive slides overlap to some extent, but the MMD^a of the material on a

^a The British workers employed the effective drop size [EDS] in place of the MMD to characterize the slides. The EDS is approximately the size below which 98 per cent of the number of particles on each slide is found and for most clouds is about 1.5 times the diameter of the mass median.¹³ⁱ

particular slide is, under favorable conditions, characteristic of that slide. Under such conditions, therefore, it is necessary only to measure the amount of material on each slide in order to obtain a rather satisfactory assessment of the mass distribution. The amount of material on a slide may be computed from microscopic counts or by chemical analysis.

The cascade impactor has a number of obvious advantages over single jet instruments such as konimeters, the Owen's jet, etc. It was originally devised and calibrated^{23,30,33,35} for the assessment of liquid particulates. For nonvolatile liquids quite precise data can be obtained if proper consideration is given to the following variables.

In the first place the MMD of the material impacted on any one slide depends to some extent on the MMD of the cloud as a whole. It depends also on the shape of the distribution curve of the cloud. Values of the MMD's on the four slides have been determined experimentally for clouds of MMD 5, 10, 16, and 100 μ .¹³ⁱ Impingement of a particle on a given slide is a question of statistical probability. The particles of a homogeneous cloud are distributed over more than one slide. Calculations have been made of the mass distribution on the slides which should be obtained with strictly homogeneous clouds.^{13m,18h} Secondly, the MMD of the particles on a slide depends on the velocity of operation of the impactor. Experimental results indicate that the MMD is proportional to the reciprocal of the square root of the flow rate.^{13j,18h}

As the result of the analysis of the counts of a large number of slides a characteristic mass distribution curve has been constructed.^{18h} By means of this curve it is possible, by chemical analysis of the amounts of material on the slides in a given experiment, to arrive at a fair estimate of the MMD's on the four slides. When an instrument has been calibrated in this way, the use of chemical methods of analysis eliminates the very tedious process of microscopic assessment of the slides.

Dust Clouds. The use of the cascade impactor for the assessment of clouds of solid particles was first investigated in this country.^{13c-i,18h} It will be evident from what has already been said that its use for this purpose is complicated by a number of factors which arise from the diversity of the characteristics of solid particles. Solid particles may be highly irregular in both shape and density. It has been found, for example, that samples of ricin prepared by the spray drying of aqueous solutions consisted largely of hol-

low spheres. When this material was further degraded by air grinding the product was chiefly in the form of thin disks. Again, the particle size distributions in dusts may be quite different from those characteristic of atomized sprays. The spray-dried material referred to was remarkably uniform in size, whereas ball-milled preparations of ricin contained a wide range of particle sizes with a large number of extreme fines. Finally, the adhesion of impinging solid particles may be incomplete and the degree of slippage may change progressively as the slide becomes coated with the agent.

These factors combine to give a wider distribution of particle sizes on a single slide than is obtained when an atomized liquid is assessed. When the particles are not spherical, the problem arises of the proper method of computing their volumes from observations of their dimensions under the microscope. Serious errors may arise if they are treated as if they were spheres. The volume of a sphere is $0.524d^3$. Heywood⁴⁴ has listed some of the factors by which the cube of the observed "diameter" should be multiplied when the particles depart from the spherical. The factor for a rounded particle is given as 0.54, for a prismoidal object it is 0.47, and for a tetrahedral particle it is 0.38. A mean value of 0.5 is suggested for a heterogeneous assembly of nonspherical particles.

Recent work has confirmed the validity of this factor for slides C and D, but it has not always been possible to apply it to slides A and B because it is on these slides that the large highly irregular and often disk-like particles are found. To measure the mean lateral dimensions of such particles and compute their volume as though they were spheres leads to an MMD for the slide which is much greater than the true value. Some attempt should be made to measure the thickness of plate-like objects and to calculate their rectangular volumes.

The frequent occurrence of aggregates in a solid particulate has proved to be particularly troublesome.³¹ The MMD of the particles impacted on a particular slide varies with the square root of the density. Since the density of a loose aggregate may be less than one-tenth of that of the unitary particles of which it is composed, it is evident that the presence of many aggregates on a slide may profoundly change the MMD of that slide. The problem of the density to be assigned to an aggregate in order to compute its mass is also a difficult one. Microscopically the best that can be done is to take a few representative

aggregates, count the number of unitary particles in them, and sum their volumes. The density may then be taken to be the ratio of this volume to the volume of the whole aggregate treated as a sphere.

Many aggregates disintegrate when they impact on a slide. They will be assessed as though they corresponded in impinging properties with the unitary particles of which they were composed, although the latter would probably not have appeared on that slide had they not been aggregated. The result will be artificially to reduce the MMD below its real value.

When unusually large particles are present in a cloud, losses may occur by impingement on the walls of the orifice of the instrument, particularly when the impactor is operated in a static cloud. Under conditions of isokinetic sampling of clouds moving with average wind velocities it has been calculated for liquid droplets that orifice losses become apparent with droplets about $50\ \mu$ in diameter and increase as the size further increases. Similar calculations for aggregates with a density of 0.1 indicate that the upper limit for reliable sampling is about $160\ \mu$. The upper limits for static clouds are probably appreciably below these figures because of increased turbulence around the leading edge of the orifice.

Summary. The emphasis which has been laid upon the evaluation of the sizes of particles on the impactor slides has tended to distract attention from the fact that the cascade impactor does not measure the size of a particle but rather its impingement tendency. Most of the difficulties in applying the instrument to the assessment of dusts have been in expressing the distribution of impacted material in terms of volumes and masses computed from microscopic observations of the dimensions of the particles. This has been a necessary preliminary to the calibration of more direct methods of interpreting the results obtained. In so far as the toxicity of particulate material is a function of the amount of material which will impact in the nose or on exposed surfaces the efficiency of a cloud should, most logically, be described in terms of its impaction factor under standard conditions. The use of particle size to characterize the cloud is a convention which may, perhaps, be discarded when instruments which measure impingement have been properly calibrated.

The MMD of slide B of the cascade impactor is close to or slightly greater than the maximum size of particles which have been found to penetrate the nasal barrier in most animals. The fraction of air-

borne material which collects on slides B, C, and D under standard conditions of operation should, therefore, be somewhat greater than the effective inhalation dose. Calibration of the instrument in such a way as to establish a relation between these two fractions should make possible an estimation of the inhalation toxicity of a cloud from a chemical analysis of the impactor slides alone.

The effective dose of a vesicant is dependent on the fraction of airborne material which is large enough to impact efficiently. Most of this fraction should be captured by slide A. The analysis of impactor slides operated in clouds of nonvolatile liquid vesicants should lead without much difficulty to satisfactory estimates of the effective vesicant dose.

15.5 PARTICLE SIZE AND TOXICITY

15.5.1 Vesicant Effects

The dose of a particulate which is deposited on an object depends upon the amount settling out plus the amount impacting.⁴⁵ The amount impacting will vary with the wind speed, density of the particle, area of the particle, diameter of the target, and nature of the surface of the target. A heterogeneous cloud of MMD $2.0\ \mu$ may have the same impactibility for a given surface as a homogeneous cloud of MMD $4.0\ \mu$.

The impingement pattern on the object will vary with particle size from a diffuse pattern with vapors and smokes to a localized (upstream surface) mosaic with coarse sprays. The volatility of the agent and the rate of absorption of the material by the target will affect the physiologically effective dose.

Preliminary indications of the order of magnitude of effect of particle size on vesicancy of a nitrogen mustard (HN3) and a nonvolatile vesicant (T) were obtained by exposures of forearms in a wind tunnel.^{13k-m,18a} At 5 mph wind speed and under conditions of temperature (about 80 F), relative humidity, and skin resistance (sweating index) such that a vapor of HN3 at a *Ct* of 1,200 mg min/m³ produces an erythema, the following tentative conclusions were reached. Smokes of MMD below $2.0\ \mu$ are less effective than vapor. A heterogeneous (atomized) cloud of MMD $2.0\ \mu$ was equally as effective as vapor. A heterogeneous cloud of MMD $8.0\ \mu$ was twice as effective but the erythema was more localized. HN3 is less volatile than mustard. T is practically non-volatile. The relation of volatility and particle size to vesicancy is illustrated by the following relationships. By topical application of single drops to fore-

arms it takes 10 times as much HN3 to produce the same skin reactions as a given amount of T.^{7,9,18b} As a 2.0- μ (heterogeneous) particulate, a *Ct* of 45 of T (area dose = *Ct* $\times$ 5 mph) is the equivalent of a *Ct* of 1,200 mg min/m³ of HN3; T is thus 27 times as vesicant as HN3. When the particle size is raised to 8.0 μ , a *Ct* of 6 to 10 of T is as effective as a *Ct* of 600 of HN3, thus demonstrating a factor of 100 or more in the vesicancy of these agents.^{18a} These findings demonstrate the importance of designing munitions which will disperse the chosen particulate in an optimum size range.

Owing to the great effect of temperature and humidity^{4,8,17} on skin reactions to given exposures, it is difficult to generalize from these data to other agents and conditions. By employing the appropriate factors for comparison of vesicant power,^{7,18h} comparisons may be made with the values given in Chapters 5 and 6. Under the conditions obtained in the experiments described in the preceding paragraphs, H vapor is about one-half as effective as HN3 vapor. The effect of evaporation of the agent after deposition on the skin has been found to be approximately the same for H and HN3,^{18a,b} despite differences in volatility. It will be indicated in the next section that of a heterogeneous cloud of T of MMD 8.0 μ , only 10–15 per cent will penetrate the human nose.

The results on vesicancy in relation to particle size apply to exposed skin areas. The presence of clothing profoundly modifies the situation. A droplet of nonvolatile agent on the surface of clothing can under some circumstances be considered innocuous, whereas a volatile agent will generate vapor which may be drawn over the underlying skin by the bellows effect of clothing. Numerous tests have been carried out on the droplet diameter required to penetrate clothing by wetting the cloth. The sizes involved are well above the particulate range considered here. Relatively few data on the penetration of clothing by small particles are available for chemical warfare agents. The amount of a particulate found on clothing is a function of filtration and impaction. A given expenditure of agent will with increasing wind speed deposit decreasing amounts on (and through) the cloth by filtration (bellows effect) but will deposit increasing amounts by impaction, especially for coarser particulates. For nonvolatile substances the amount penetrating cloth by impaction forces appears to be a small fraction of the amount penetrating by filtration.^{18b}

For nonvolatile materials there is definite disadvantage to the use of particulate clouds coarser than 1 to 2 μ in diameter if penetration of clothing is to be achieved. Increased turbulence at higher wind speeds appears to reduce the percentage that penetrates by filtration.^{18b}

15.5.2 Penetration of the Nose

Initial experiments were designed to determine the particle diameter at which 50 per cent of the mass of a given cloud passes the nose.^{13k,26–29} Values obtained on four human subjects are given in Table 2.

TABLE 2. Penetration of the human nose by particulates.

Agent	Density (g/ml)	Flow rate (lpm)	Diameter for 50 per cent penetration (μ)
Corn oil	0.93	17	5.6
		60	1.8
Dry NaHCO ₃	2.2*	17	2.1
		60	0.8

* Actual density in nose somewhat lower because of hydration of particles.

These experiments were extended in an attempt to determine the percentage penetration of the nose at various sizes for materials of differing physical characteristics, e.g., liquid corn oil of density 0.9 and dry NaHCO₃ of density 2.0, and at various rates of breathing. The results are presented in Figures 1 and 2. It is of interest that there is little difference in

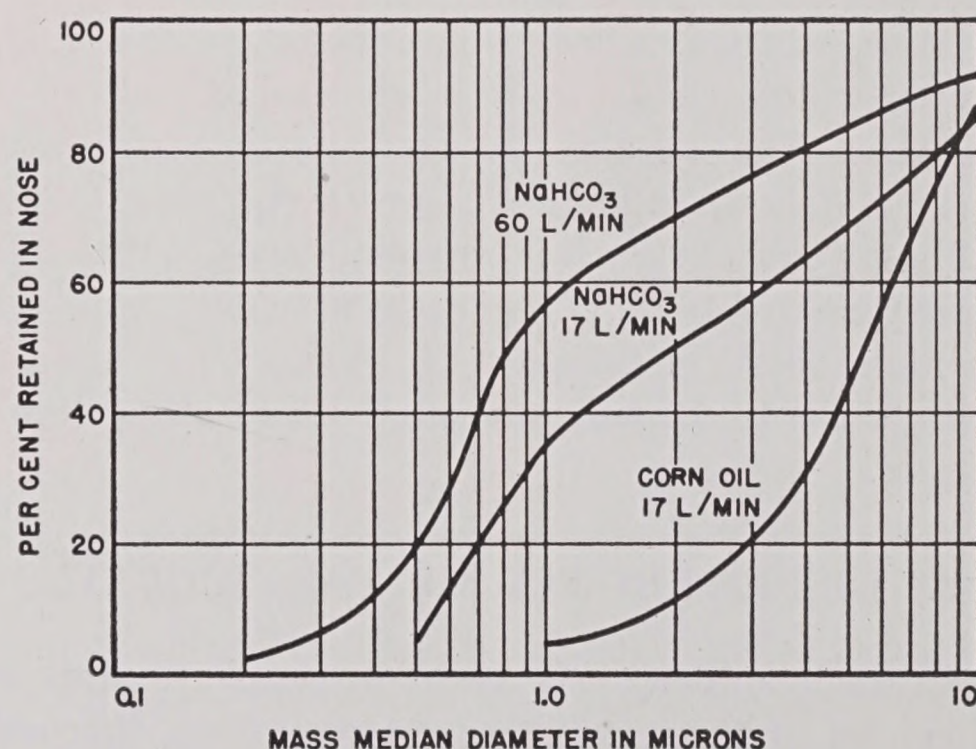


FIGURE 1. Nasal retention of particulates in man.

nasal penetration between flow rates of 17 and 29 lpm (unpublished data). A change from 17 lpm to 60 lpm changes the value for 50 per cent penetration from 2.1 to 0.8 μ . Regardless of flow rate or density, parti-

cles $10\ \mu$ in diameter have approximately a 10 per cent chance of penetrating the nose.

Over the size range shown in Figures 1 and 2 it would appear that for a given particle diameter the

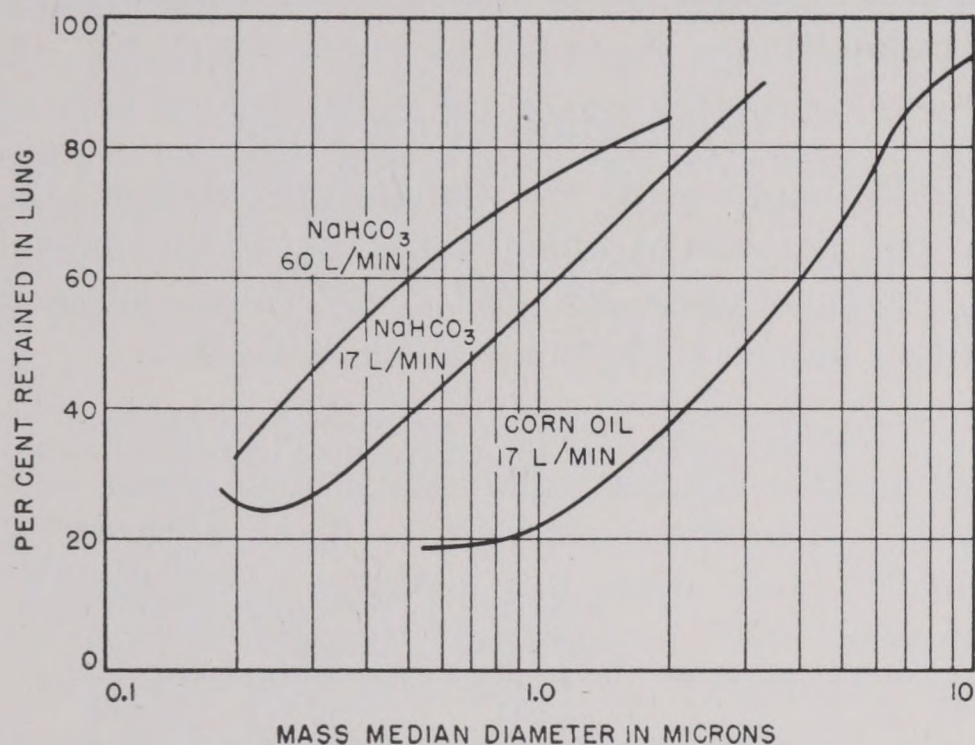


FIGURE 2. Lung retention of particulates in man.

lung retention is about 20 per cent more efficient than the nose. From another viewpoint, the same efficiency in retention obtains for particles in the nose which are 2.5 times the diameter of those in the lung.

When molecular dimensions are reached the nasal and lung retentions increase above those found at $0.2\ \mu$.^{18i,29}

15.5.3 Inhalation Toxicity in Animals

Mice, rats, and rabbits were exposed, while at rest, to particulate clouds of W in glycerol at controlled MMD's. The relation between particle size and $L(Ct)_{50}$ is shown in Figure 3. For the size range 0.5 to $7.0\ \mu$ the effect is much more pronounced in rats and mice than in rabbits. These data, which are reviewed in more detail in Chapter 12, should be compared with those of British authors²⁹ using other techniques.

15.6 DISPERSIBILITY OF PARTICULATES

In the laboratory it is relatively simple to prepare clouds of unitary particles by atomization, thermal generation, or in electric arcs. Previously comminuted powders may also be dispersed largely as unitary particles in special apparatus. Munitions capable of dispersing previously comminuted powders in the unitary state have yet to be developed. Powders differ in ease of dispersibility, as shown in various

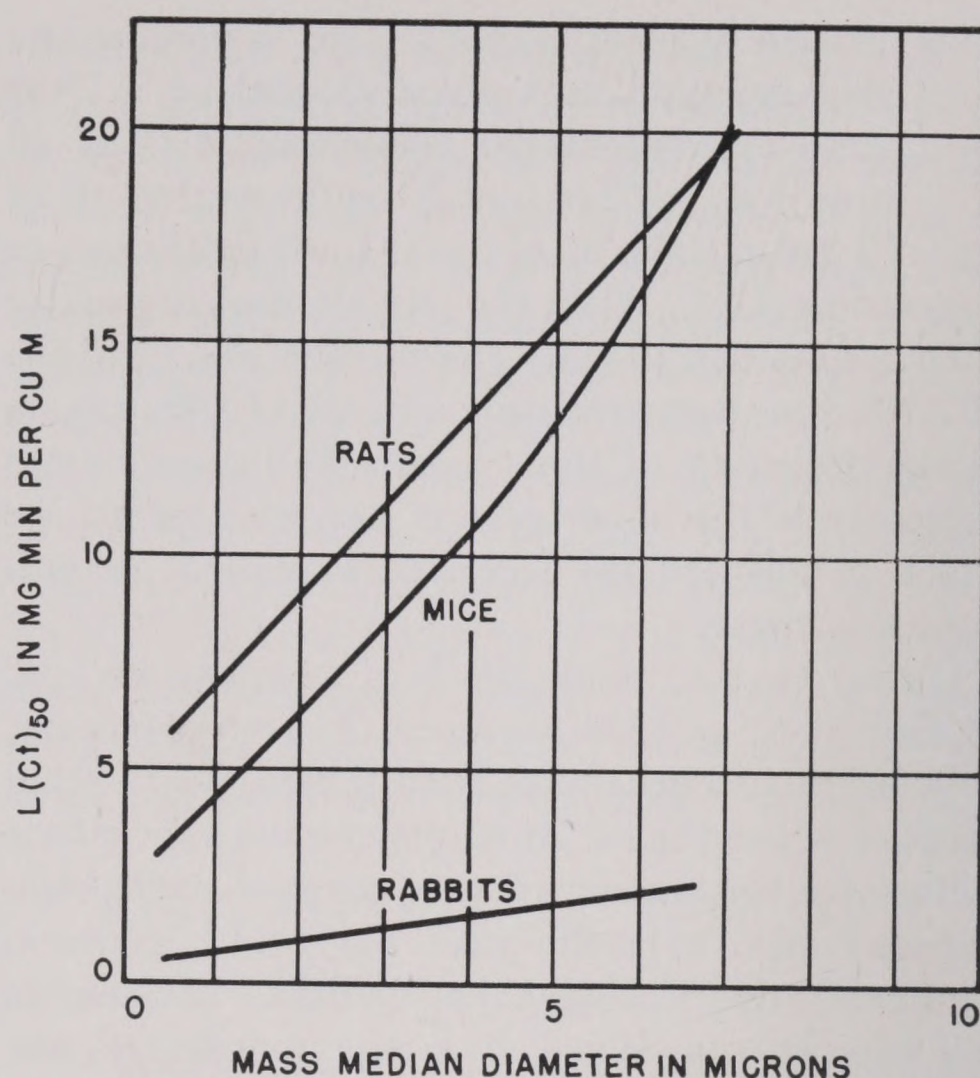


FIGURE 3. Inhalation toxicity of ricin in relation to particle size.

laboratory tests. Such tests are, however, generally meaningless in terms of dispersibility by field munitions. The factors involved in field munitions which are difficult to scale up from laboratory tests include aggregation phenomena occurring prior to, at the time of, and immediately subsequent to dispersal. These aggregation phenomena are influenced by geometry, strength of materials, brisance of explosives, and scale of munition.^{21,22}

To date field experiments³⁹ have, however, almost universally confirmed the finding³⁵ that suspensions in organic nonsolvent media result in much higher dispersion efficiencies than can be obtained by use of gas ejection munitions or standard munitions with dry fillings. Owing to low bulk density of dry fillings, suspensions permit a higher ratio of active filling to munition weight.

15.7 FIELD ASPECTS OF PARTICULATE ASSESSMENT

The outstanding observation resulting from field experiments on dispersion of previously comminuted powders by field munitions is the fact that the fraction of material airborne in the size range of the original filling is generally insignificant. Most of the mass of the filling appears in a highly aggregated

state. Field sampling must not only evaluate the gross clumping and spillage but also account for the low toxicity (in terms of chemical *Ct* or area dose) of the more lastingly airborne clouds. The frequently occurring light fluffy (snowflake) aggregates are generally not encountered in laboratory investigations. They are particularly deceptive since they may be readily disrupted in the sample and thus appear as a group of component unitary particles.

The orifice velocity of field sampling equipment should not deviate markedly from the wind speed if particles above 50 μ in diameter are to be readily sampled. Where power-operated devices are employed, the requirements of pump capacity for appropriate sampling of particulates, which are higher than for vapors, may cause some embarrassment.

In early experiments fine wires were tested as sampling devices but discarded because of the differing impaction efficiencies for small and large particles.³⁵ By employing wires or tubes of three different diameters, however, the relation of collection efficiency to wire diameter can be utilized to calculate particle size and area dose from the mass of material collected on each size of wire.^{18a,c,d,45} This device dispenses with power requirements when sampling in wind speeds above 3 mph. At 1 mph corrections for settling are required.

There are marked difficulties in the assessment of initial clouds containing vapor and particulate concentrations. The chemical drop trap²⁵ and the chemical selector³⁴ indicate possible methods to be developed. The use of impingers³⁶ or filters is recommended when numerical values of the MMD or impactibility are not required. The total chemical *Ct*, without regard to size, can be measured for dry particulates with filters. Rayon-asbestos, esparto-asbestos, and gas mask filter papers may be used. For use with W these papers are unsuitable owing to the strong adsorption of the protein on the paper. Cellulose acetate filter batts¹² do not absorb proteins and in addition are soluble in appropriate organic liquids. Another qualitative device for evaluation of aggregates is the "sticky finger."^{14a}

Methods have been developed and results obtained during the period 1941 to 1945 which indicate the desired particle size for various purposes. In the same period, however, no adequate munition capable either of producing such sizes or dispersing materials already prepared at those sizes has been developed. Thus, at the date of writing, W (which has in the laboratory several score times the toxicity of phosgene) has (in the field) been found to be only seven times as toxic as phosgene in the best munitions available.³⁹

Chapter 16

APPARATUS AND TECHNIQUES UTILIZED IN TOXICOLOGICAL STUDIES ON CHEMICAL WARFARE AGENTS

By *H. A. Wooster* and *W. L. Doyle*

16.1 INTRODUCTION

IN THIS CHAPTER are summarized methods developed and utilized for toxicological studies at the University of Chicago Toxicity Laboratory [UCTL]. Pertinent contributions of other NDRC Division 9 contractors are included, but no attempt is made to review systematically developments made by other agencies.

The apparatus and methods are described under the following major headings: (1) gassing chambers, (2) methods of dispersing agents into chambers, (3) sampling equipment, (4) precision methods of testing inhalation toxicity, and (5) methods of testing vesicants. Each section starts with a discussion of the relevant principles and is followed by a brief description of specific items of equipment and procedure, together with an evaluation of the merits and limitations of each. Descriptions and construction details for the more important items of equipment will be found in the reports listed in the Bibliography and referred to in the text.

The work leading to the development of apparatus included in this report was initiated prior to March 15, 1945, at which time the contract with the University of Chicago was assumed by the Chemical Warfare Service. Subsequent work has been reported where it was in logical extension of apparatus initiated under the prior contract.

16.2 GASSING CHAMBERS

16.2.1 General Description of Design

The earliest form of gassing chamber was a closed container in which the animals were placed and the agent dispersed. Despite the simplicity of such an apparatus, its use introduces many complexities. The actual concentration of agent in it at any one time is a result of the action of at least two variables — the rate at which the agent is sprayed into the chamber and the decrease of the concentration. The latter is influenced in several ways — absorption on the chamber walls, chemical changes of the agent

(the hydrolysis of dichloroalkyl arsines, for example), and, in the case of particulates, aggregation of the smaller particles. Animals kept in a closed chamber for any period of time may change the carbon dioxide content of the air sufficiently to distort their respiratory patterns. Nominal concentrations in such chambers are almost meaningless, and analytical concentrations are difficult to interpret.

Lehmann, in Germany, in a long series of investigations (1884–1913) studied the effects on animals of various toxic vapors used in industry. His method was to expose animals in a modified Pettenkofer respiration apparatus to a continuous flow of air containing a constant and known concentration of the agent being studied. Almost all the gassing chambers used in this country since 1918 are based on this constant flow, or “dynamic” principle. (It should be noted that English workers, in many of their screening runs, employed “static” chambers during World War II.)

The ratio of chamber volume to air flow is critical in the design of such chambers. Silver²³ derives the basic equation covering chamber equilibration times:

$$t_{99} = 4.6 \times \frac{a}{b}$$

where t_{99} = time for the chamber concentration to attain 99 per cent of the theoretical nominal concentration.

a = volume of the chamber in liters.

b = the rate of air flow in lpm.

It will be seen from this that a chamber having an air flow of 1 chamber volume per minute will come to equilibrium in about 5 minutes; with 10 chamber volumes per minute equilibrium is attained in 0.5 minute. A quick equilibration time has several advantages — momentary changes in concentration, such as those produced by the introduction of animals, are quickly rectified, and unstable materials have less time in which to decompose. The saving in material by the use of a shorter equilibration time is overbalanced by the larger amount of material necessary to set up

a given concentration, but a 5-g sample is generally adequate for a single test of a substance toxic at 0.3 mg/l. When slowly volatile materials, which exist as both vapors and aerosols, are dispersed in chambers of very high flow rates, such as the auxiliary chamber for the 200-l medium flow chamber (see below), effects of the flow rate on toxicity may be encountered.

The flow of air through the chamber may be produced by either positive or negative pressure. In most chambers negative pressure is used, to minimize the tendency for toxic materials to escape into the laboratory. Standard equipment for this is a gear-type (Roots) blower V-belted to an electric motor. An air ejector is used on the chamber for the large Benesh atomizer, and water aspirators have been used on some small smoke chambers. Positive pressure has been used on three chambers — a large screening smoke chamber, a small chamber used for testing the toxicity of gasoline, and the microline. In these chambers the air flow is controlled by the volume of air blown into the chamber.

Standard equipment for measuring air flow through the larger chambers has been an orifice in a Monel plate in the effluent line between the chamber and the filters. A differential manometer, filled with butyl phthalate, is connected to each side of the orifice. To calibrate such a flowmeter a large dry-type gas meter is connected to the chamber and all other openings are sealed. A working calibration chart is prepared from these readings. The dry meter is calibrated by positive displacement of a measured volume of air.

The standard orifices are about 0.8 inch in diameter. Because of their location they are subject to contamination and corrosion. When aerosols are used in the chamber they tend to clog up the hole and make it smaller. It is advisable to recalibrate such orifice flowmeters at least once a year. A much larger orifice (about 2.5 inch) has been used in the chamber for the large Benesh atomizer, which was designed specifically for use with smokes. An inclined differential manometer is necessary to read accurately the small pressure gradient resulting from the use of such a large orifice.

The effluent from these chambers contains a large proportion of the original toxic material. It is passed through replaceable charcoal filters. When nonvolatile vesicants have been used, the filters become heavily contaminated, and their removal and recharging is a hazardous procedure. The effluent from

all chambers plus the effluent from all rooms is drawn off by rotary blowers and discharged into a large incinerator stack. The dilution afforded by the stack provides a larger margin of safety and in some cases permits dispensing with charcoal filters. The UCTL stack has an average inside diameter of 16½ feet and is 100 feet high. Under normal conditions the stack discharges 750,000 cfm.

The chambers are of metal and/or glass construction. The all-metal chambers are constructed of welded 3/16-inch mild steel plate, which is protected on the inside with a baked-on vitreous or bakelite resin (Lithcote) enamel. Connections to these chambers are made with standard plumbing pipe fittings. Ten-liter wide-mouth glass bottles with holes drilled in them have been used for several small chambers. The chamber on the small Benesh machine is made entirely of triplex safety glass, cemented together. Composite structure is represented by the 400-l chamber, made from a length of Pyrex industrial pipe 12 inches in diameter, fitted with brass ends, and the 488-l chamber, made of metal lined with plate glass.

One of the more important procedures in gassing animals is the method of introduction of animals into the chamber. The simplest method, which is entirely feasible with mice, is to open a port and insert the caged animals. This is routinely done with the small smoke chambers and with some of the larger chambers which have small auxiliary ports on their larger doors. With larger animals, some sort of sliding carriage for the cages must be provided. This is, in essence, a three-sided box, the ends of which are plates, and the bottom an open structure. The side view can be represented by . Either end may serve as a closure for the opening in the chamber side. When such a carriage is rapidly pushed into a chamber, a certain piston action is exerted. The carriage on the big Benesh machine was designed to avoid this. When the carriage is out of the chamber, closure is provided by a vertically sliding glass panel. Thus the carriage needs only the form . In high-flow chambers the animals may be placed in the chamber before the agent is put in. This is practicable because of the short equilibration time of such chambers. However, it should not be used for short exposures to substances which deviate markedly from Haber's law.

One difficult problem in the design of chambers is the position of the port through which the agent is to be introduced. This may be at either the top or

the side of the chambers. The top is a somewhat more convenient location, inasmuch as all the paraphernalia connected with dispersal may be placed on top of the chamber out of the way. When dealing with gases or with aerosols set up by a baffled atomizer, the position is not so important as with other degrees of dispersion because the materials enter the chamber at a low velocity and loss by impaction on inlet tubes is negligible. With concentric atomizers dispersing semivolatile materials, introduction from the top means that the spray must undergo a right-angle bend to get into the chamber, with consequent loss on the mixing bowl. A jet fed in from the side of the chamber must be aimed with care to clear the animal cages. It would seem advisable to design future chambers with provision for the optional use of either route of entry.

Little attempt has been made so far to control the temperature and humidity of air entering the chamber. In most cases the chambers withdraw air from the laboratory and operate at the ambient temperature and humidity. In the microline provisions were made to humidify the entering air. Some small chambers have been operated in a thermostated water bath. The most elaborate regulation is in the man-chamber, which has automatically controlled equipment for heating or cooling, and varying the water content of the entering air, as well as temperature control of the room surrounding the chamber.

16.2.2 Description of Specific Chambers

RECTANGULAR CHAMBERS LARGER THAN 200 LITERS

400-Liter Standard Chamber.^{6,23} The first large chamber used at UCTL was built from a design standard at Edgewood Arsenal. This chamber is fitted with a sliding carriage 8 inches high and 15 inches wide. This, at most, can hold 4 cats or rabbits, or 20 guinea pigs or rats. The chamber air flow can be regulated between 50 to 90 per cent of the chamber volume per minute. A wooden sliding carriage with stocks for surrounding the necks of exposed animals was made to study body and head exposures.

In use, this chamber was found to have several limitations. Animals larger than cats could not be exposed routinely (single dogs were used in body exposures). Appreciable difficulty was encountered in working with lewisite, owing to wall loss at the low flow rates — e.g., the nominal LC_{50} of lewisite for mice was approximately three times as high with the standard chamber as with the Benesh machine.

*880-Liter Standard Chamber.*⁶ This chamber is

identical in principle and operation with the 400-l chamber. The sliding carriage is somewhat higher in relation to the height of the chamber. Its cross-sectional dimensions are 23x55 inches. This gives it a maximum animal capacity of 4 small dogs, or 2 large dogs, or 1 or 2 goats, or 6 monkeys. A mixed group of 1 small dog, 4 rabbits, 4 cats, 10 guinea pigs, 10 rats, and 20 mice can be exposed at the same time.

At a later period a small door was built into the outside plate of the sliding carriage, making it possible to put small animals into the chamber without pulling out the carriage.

This chamber has been calibrated with mustard gas (H), using the Northrup titrimeter.²¹¹ The air flow was 700 lpm, and the nominal concentration may be expected to be in error by about ± 5 per cent. The concentration built up is the same in all parts of the chamber within 1 per cent and the drop in concentration on moving the carriage in or out is probably not more than 5 per cent.

This chamber has, perhaps, been the most consistently useful for general work.

*200-Liter Medium Flow Chamber.*¹⁴ This chamber was designed to provide a chamber in which dogs and other large animals could be exposed to agents at rates of chamber exchange comparable to those at which mice had been exposed in smaller chambers. By interchanging a glass door on the side of the chamber for one which is provided with platforms and head stocks, mice, rats, or guinea pigs may be exposed to gases either by inhalation or by body exposure alone. A similar arrangement can be attached to the front carriage for similar exposures of cats, rabbits, or dogs.

Some time after the chamber was built an auxiliary high-flow chamber was added.¹⁴ The new chamber was built onto a removable side plate which could be substituted for the side door. The cross-sectional diameter of the high-flow chamber is about one-ninth that of the main chamber. Air is drawn from the main chamber into the auxiliary chamber and thence into the exhaust line. When the chamber is operated at 500 lpm, the velocity is increased to 3 mph just before the toxic agent reaches the animals, with a minimum velocity of 0.5 mph in the center of the compartment in which the animals are exposed. These velocities may be increased or decreased by varying the air flow. The incident velocity may be changed by changing the size of the slits through which the air stream enters.

The high-flow chamber is 18x7x3 inches. It

is divided into three compartments by two longitudinal walls, each of which contains 10 slits, $2 \times \frac{1}{2}$ inches. The slit size may be varied. The compartment in which the animals are exposed is $18 \times 3 \times 3$ inches, and is located between the other two compartments. The long, slender compartments on each side have openings in the floor through which analytical samples may be drawn. The inner of the small compartments is open on the side communicating with the main chamber, and on the distal side has the slotted wall openings. It serves as a mixing chamber to insure that all the animals are exposed to the same concentration. The outer of the two compartments has a slotted inner wall through which the air stream leaves. The effluent is carried away through an opening in the end of this chamber. Analytical samples can be drawn before and after the agent passes the animals.

Animals may be exposed by total exposure, or by body or head exposure alone. A special manifold is provided for the last two types. The lower portion of the side plate to which the high-flow chamber is attached can also be used for either body or inhalation exposures at low flow rates. Total exposures for low flow rates can be carried out by placing animals in the main chamber. Animals may, therefore, be exposed simultaneously to high and low flow rates either by total exposure, body exposure, or inhalation exposure.

The 200-l chamber differs in several design details from the standard chambers. The carriage is provided with castors, making it more convenient to slide it in and out. In the standard chambers the bare metal of the door seats against the bare metal of the chamber. In this chamber sponge rubber gaskets are provided. The toxic agent is usually admitted at the top of this chamber instead of at the side.

In the use of this chamber a good agreement has been obtained between analytical and nominal concentrations. $L(Ct)_{50}$'s obtained by this chamber correspond with those obtained in the small high-flow chambers. This is not the case with values obtained from the 400-l standard chamber.

429-Liter Glass-Lined Chamber. This chamber was designed specifically for use with aerosols. It is lined with plate glass. The sliding stainless-steel animal carriage is attached to a glass panel which forms the front wall of the chamber when the carriage is in place. When this is not used a counterweighted glass panel drawn down from the top seals the chamber. Interlocks are provided so that the carriage cannot be

pushed in until the sliding panel is fully raised. This scheme is a trifle complicated and requires two operators for rapid action, but has the advantage that it does not exert the plunger effect of the usual chamber carriage. A small circular auxiliary port in the panel on the carriage permits caged mice to be placed in the chamber without opening the main door.

A large air injector is used as a pump to exhaust air from the chamber. This gives a maximum air flow of 900 lpm at 35 lb air pressure. An inclined differential manometer, reading across a large orifice, gives the chamber air flow. Such a large orifice is less sensitive to fouling than those commonly used. The air injector is also less subject to fouling with aerosols than gear-type blowers. Variations in the pressure of the air running the Venturi are corrected by a diaphragm-actuated regulator.

The glass lining makes this chamber particularly easy to clean. It is much quieter in operation than the mechanically driven chambers.

This chamber has been calibrated with H by means of the Northrup titrimeter, at a nominal concentration of $38.8 \mu\text{g/l}$ and an air flow of 1.5 chamber volumes per minute.^{21g} The following conclusions were drawn:

1. The Ct calculated from the nominal concentration will be in error by about $+16$ per cent.
2. The 10-minute Ct calculated from an analytical concentration measured at about the mid-point of a 10-minute exposure will be in error by about ± 3 per cent.
3. The Ct calculated from an analytical concentration based on a sample drawn over the entire period should be in error by less than 3 per cent.
4. In general, errors caused by the fall in concentration that occurs upon pushing in the animal cages may be neglected for 10-minute exposures and can be corrected by analytical samples drawn at intervals during the entire exposure period.

Screening Smoke Chamber.^{3,16} This chamber was designed for the repeated exposures of animals to low concentrations of agents employed as screening smokes. It was made large enough for monkeys to live in and was fitted with automatic controls. The chamber is 4 feet square and 7 feet high. Its volume is 3,078 l. The base is a concrete block fitted with a drain and lined with sheet metal. The top is wooden, as are the corner posts. The sides are of glass. A common wooden door with a glass panel is let into one side. This door is weatherstripped. The whole structure is lined with a very heavy wire mesh.

Two fans are used with this chamber. A continuously running exhaust fan provides ventilation. An intermittently operating centrifugal blower giving 2,180 lpm is mounted on the top of the chamber. Just below the ceiling outlet is a suspended baffle plate. A six-jet atomizer (DeVilbiss experimental model No. 7030-1), connected to the compressed air line, discharges into the inlet of the centrifugal blower. A General Electric time switch controls the solenoid valve, feeding compressed air to the atomizer and the relay actuating the inlet fan. These go on and off together, in a cycle of 30 minutes on and 30 minutes off.

The chamber was found to come to equilibrium in 10 (± 2) minutes. This is somewhat longer than the theoretical time. Forty-five per cent of the equilibrium concentration is reached after 1 minute, and eighty per cent at 5 minutes.

Lacrimator Chamber.^{21a} This chamber is essentially a 400-l standard chamber with a maximum air flow of 1,000 lpm. The adaptation for use with lacrimators consists of three ports projecting from the center of the chamber walls on three sides. Eye pieces, which fit the ports snugly, consist of rubber diaphragms edged with rubber tubing. Swimming goggle frames are cemented around holes cut in the diaphragm. The sternutator provision consists of industrial-type nose and mouth respirator masks connected to the chamber with lengths of gas mask hose. There are six of these.

The subjects signal their response by tapping keys, located under the ports, which cause signal magnets to mark an automatically timed rotating kymograph. The subject taps the key when irritation is first experienced, and again when he feels tears starting to form. Thereafter he depresses the key each time he is forced to close his eyelids and releases it when the lids are once again open. At the end of the run there is a graphic record of the onset of irritation and of lacrimation, as well as of the periods during which the eyes were open or closed.

Owing to the low priority assigned to lacrimators and sternutators, this chamber was never extensively used or completely calibrated.

Great Lakes Man-Chamber.^{211,26,27a,g} This chamber was designed for the exposure of human subjects under conditions of temperature and humidity controllable by the investigator and independent of ambient conditions.

The chamber is made of $\frac{3}{8}$ -inch boiler plate, lined with $\frac{5}{16}$ -inch sheet lead. Its volume, exclusive of

the air lock, is about 17,300 l. The maximum flow rate through the chamber is about 5,100 lpm. All control of concentration (H has been the only agent used) is done with the Northrup titrimeter, so that exact values for this flow are not so necessary as when an attempt is made to estimate the nominal concentration.

This chamber is equipped with automatic pneumatic controls for temperature, relative humidity, rate of flow, and pressure. They function as follows:

1. All air coming into the chamber passes through a commercial air-conditioning unit. It emerges from this into the chamber at 26 F, saturated with water vapor. When warmed to 70 F, this air is at about 35 per cent relative humidity. The temperature and relative humidity of this air represent the lowest levels at which the chamber can be operated.

2. The desired wet bulb and dry bulb temperatures are set on the controlling-recording apparatus and the steam lines are opened. Heating is controlled by a steam coil controlled by the dry bulb temperatures. Lowered wet bulb temperatures cause the automatic humidity valve to open, injecting steam into the chamber. When the wet and dry bulb temperatures reach the desired values, the humidity valve closes and the by-pass dampers open; thus the incoming air is conducted underneath the heating coil rather than through it.

3. When the air is pulled through the heating coil, there is more resistance in the system than when the air is by-passing the coil, so that adjustments of the flow are necessary. This regulation is controlled by dampers on the discharge side of the exhaust fan. When the flow rate drops below 5,600 lpm, these dampers open and permit more air to be drawn out of the chamber; as the flow rate rises, the dampers close and cut down the flow. The flow rate usually oscillates between 5,300 and 5,900 lpm.

4. Ordinarily the fluctuations in the amount of air discharged would produce variations in the pressure inside the chamber. Such variations are eliminated by automatic control of the dampers on the discharge side of the supply fan. The pressure controller is set for a differential of 0.1 inch of water; when the pressure in the chamber increases, the control damper effects an opening of the dampers to the room, so that less air is passed into the chamber. Similarly, when the inside pressure falls to a value lower than 0.1 inch of water below the outside pressure, the dampers close to permit a larger volume of air to enter the chamber.

An air lock is equipped with motor-driven ports by means of which fresh air may be diverted through the air lock when men wearing contaminated clothing are leaving the chamber.

Measurements of the wind speeds in the chamber showed that the velocities vary from less than 0.4 mph in the corners to over 8 mph in front of the fans, with an average of 2.5 mph for 32 positions.

CONSTANT-FLOW CHAMBERS SMALLER THAN 100 L

*The Microline.*¹ The 400- and 800-l standard chambers were found to be unsuited for "screening" new agents of which only small amounts were available, and for working with unstable substances such as the arsenicals. The microline together with its ancillary chambers was designed to provide a small chamber through which a relatively high flow of air at controllable humidity could be sent.

The influent air is delivered via two parallel series of bubblers and absorbers. One of these delivers dry air, the other saturated. These are mixed in the desired proportions and passed through a dispersing bubbler or an impinging atomizer containing the agent and thence into the chamber.

The first chamber used with this microline was a 10-l screw-capped wide-mouthed bottle. A cylindrical cage fastened to the bakelite screw cap contained six mice. A U-shaped manifold, both ends of which passed through the screw cap, was used for body exposures. The heads of mice were stuck through holes in the manifold while fresh air was circulated through it, and a concentration of toxic agent was set up in the chamber. A branched manifold for testing toxicity by inhalation could be substituted for the chamber. This enabled 8 mice to inhale the agent while their bodies were exposed to room air.

These chambers and manifolds had several drawbacks. Only mice could be used in the chamber, and not more than six of these. The agent flowed linearly through the chamber, so that if the first animal affected the composition of the agent the last might get a lowered dose. The inhalation and body exposure manifolds could hold only 8 and 6 mice, respectively, and were difficult to manipulate.

Later a commercial Letrodryer unit⁸ was installed to supply adequate amounts of dry compressed air. The size of the water-saturators was increased proportionately. An 11.5-l chamber was constructed of plate glass cemented together and supported inside of an angle iron framework. This was designed to assure equal distribution of the toxic-air

mixture directly to each animal. To do this the material is conducted into an H-shaped channel, each arm of which has an opening connected with a slit in the glass side of the chamber, 0.1 mm wide and extending from front to back. The channel is designed to give uniform flow through the whole length of both slits, which form two horizontal lines on each side of the chamber and are so centered that, when the mouse cage is placed in the chamber, the animals are directly opposite the slits in line with the flow of the material. This permits a high degree of uniformity in exposure of the mice. The effluent is carried off by an identical arrangement on the other side of the chamber. This chamber has a capacity of 20 mice, 3 rats, or 3 guinea pigs.

A body exposure manifold which holds 20 mice and fits into the chamber and a separate inhalation manifold holding 16 mice have also been constructed.

Using an aerosol of HN3, recoveries of about 65 per cent were obtained from the chamber in the absence of animals and of about 75 per cent from the inhalation manifold. Recoveries of more volatile materials are well above 90 per cent.

Small Smoke Chamber. This was essentially a vastly simplified microline. Dry air was passed through a dispersing bubbler or impinging atomizer. The reducing valve on the compressed air tank, with the atomizer connected, was calibrated in liters per minute versus pressure. Auxiliary air could be bled in through a Y tube to bring the flow to the desired value. The air flow was then led through a water or steam jacketed condenser into the wide-mouthed bottle used as a gassing chamber. The toxic material was blown out of the chamber into the air of the hood in which the whole setup was placed. This chamber has been used in a thermostated water bath for exposures above or below room temperatures.

This setup, which required a minimum of apparatus, proved to be quite useful for screening materials of low vapor pressure. Its use was limited to materials which would melt without decomposing, so that they could be dispersed with an impinging atomizer. Mice, guinea pigs, and rats were the only animals that could be fitted into the chamber.

A modification of this chamber was used to set up very high concentrations of gasoline vapor.^{21g} All air entering the chamber was blown through a concentric atomizer, and then passed through a steam-heated Friederich's condenser. A trap in the line removed nonvolatilized material before it entered the chamber.

SPECIALIZED CHAMBERS

The Explosion Chambers. Chambers were required to assay the action of high explosives on the toxicity of certain chemical warfare agents. It was necessary to construct a rugged chamber in which small amounts of explosives could be set off and the toxicity of the resulting airborne material assayed.

The first of these was a 1-ton shipping container for war gases such as mustard. A port (12-inch diameter) was welded on this. It was fitted with a steel cover 2 inches thick, bolted down with 1-inch bolts. This chamber was used while a specially designed chamber was being built.^{21a}

The latter was constructed from 18-8 stainless steel. The interior is polished to a No. 4 finish. The vessel is 48 inches in outside diameter and approximately 8 feet high. The volume is 2 cu m. It is mounted over a concrete pit in a specially constructed laboratory and is shielded by heavy concrete walls. The dome-shaped top of the chamber is held down by 80 bolts under spring tension to act as a safety valve yielding at 80 psi. Easy access to the interior is provided by an 18-inch manhole with single-screw closure. There are eight 4-inch ports which can be closed with $\frac{3}{4}$ -inch Pyrex or stainless-steel plates; additional ports are provided for valves and electrical leads. The chamber is equipped with a shower for flushing. The walls and interior fittings are designed to permit complete drainage to the valve at the bottom. This permits maximum recovery of the products.

Steam lines lead to the chamber for decontamination. The residual gases in the chamber may be drawn through a 200 cfm collective protective canister.

When metal bombs are exploded they are surrounded by stainless-steel baffles to protect the walls of the chamber. This is not necessary for glass or plastic bombs. The resultant gas-smoke mixture is drawn through Pyrex glass piping to a small glass constant-flow exposure chamber. The effluent from the exposure chamber is filtered and absorbed in the usual fashion.

This chamber is somewhat small for testing the effects of high explosives on chemical warfare agents. Twenty-five grams of explosive is the maximum that can be detonated. It would be desirable to have means of heating and cooling the chamber walls. Other than this, the chamber has proved quite satisfactory. It is the only known explosion chamber permitting recovery and analysis of the entire residue.

*The Wind Tunnel.*²¹ⁱ The UCTL became interested in the relation of particle size to vesication on bare skin and through clothing and in the relative efficiencies of the vapor and aerosols of the same material as vesicants. It was necessary to construct a wind tunnel in which the arms of human subjects could be exposed to airborne agents moving at various and variable velocities.

The tunnel, circular in cross section, is 14 feet long and $2\frac{1}{2}$ feet in diameter in the largest places. It is fashioned after certain Porton models³⁰ designed to give an even distribution of droplets across the working section. It differs from streamline tunnels, in which markedly higher velocities exist at the center of the stream than at the edges.

The wind tunnel proper (Figure 1) is of cylindrical cross section. Two truncated cones (*B* and *C*) are placed base to base with a short base diameter cylinder in between. This assembly precedes a longer cylindrical section (*D*), 18 inches in diameter and 3 feet long. The source of vapor or particulate spray is an atomizer or bubbler orifice located at the mouth of the tunnel. In order to mix the narrow plume of agent with the main air stream, the diameter of the tunnel is increased (*B*) to produce turbulence. The expanding cone is followed by a reducing cone (*C*) to give approximately constant velocity across the stream in the cylindrical working section (*D*). The flow through the working section is somewhat turbulent at 7 mph. This turbulence can be decreased by placing a hardware cloth screen in the reducing cone, but such a screen causes an increase in concentration of the larger particulate droplets in the center of the stream. Without the screen the droplet distribution is quite homogeneous across the tunnel. Turbulence creates vortexes in the working section, producing differences of about 10 per cent (at 7 mph) in the velocities at opposite sides. This difference could probably be decreased by increasing the length of the cylindrical section between the two cones *B* and *C*. With particulate clouds of nonvolatile droplets in which 30 per cent of the mass is in the size range 10–30 μ , there is a just perceptible loss on the walls; the loss is negligible with smaller droplets. With droplets of $150 \pm 50 \mu$ in diameter there is a slightly greater loss on the bottom of the tunnel than on the top. Most of the loss occurs in the reducing cone (*C*).

The source of suction is the room ventilation which leads via filters to the incinerator stack. The flow is regulated by adjustable louvers. To obtain velocities above 25 mph a tube with its own reducing cone and

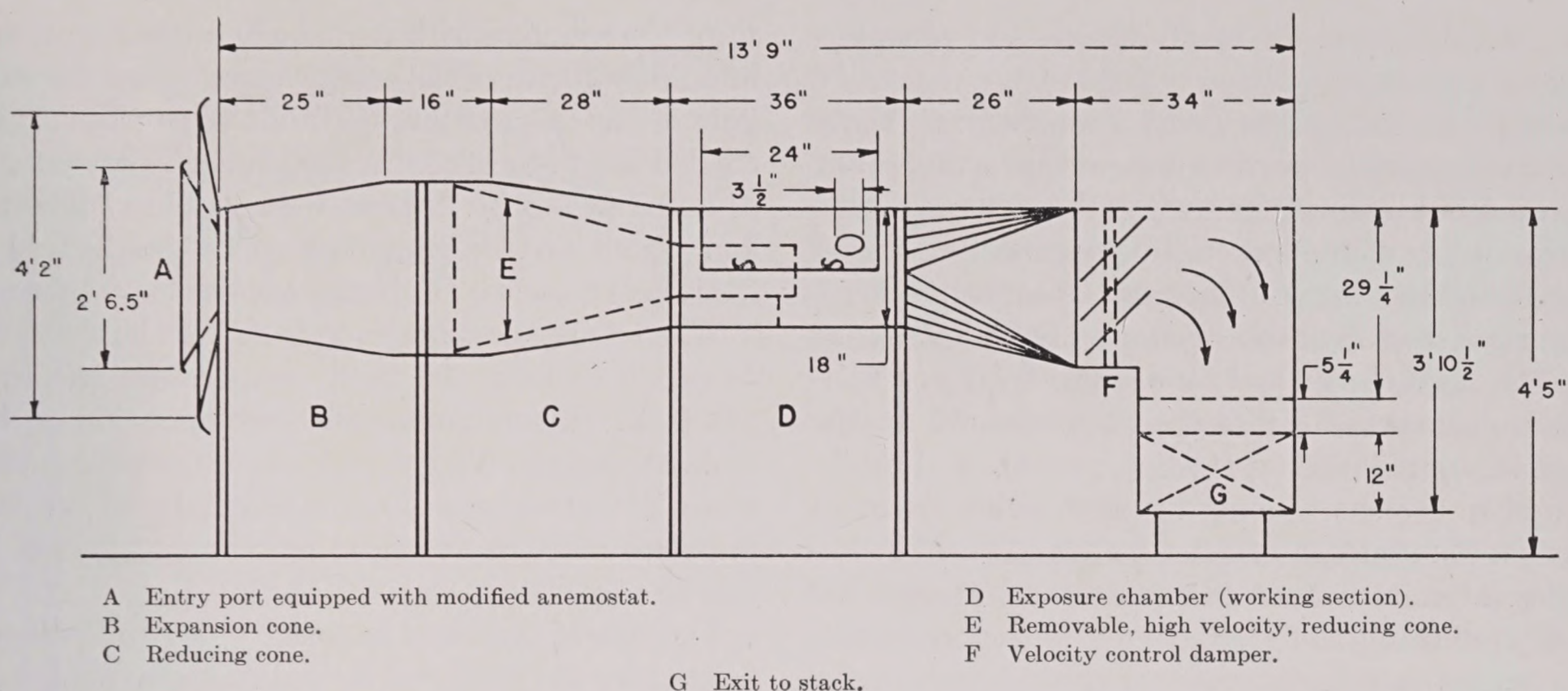


FIGURE 1. Wind tunnel, elevation.

smaller working section is available. Fairing of the incoming air stream is accomplished with a commercial "anemostat" with the three central vanes removed. The working section is provided with a door, windows, and sampling ports for introduction of animals, arms, and instruments. Air speeds are measured with a commercial Velometer.

The wind tunnel has been employed in studies on vesication by particulates and in the development of methods of assessment of particulates (see Chapter 15).

16.3 METHODS OF DISPERSING AGENTS INTO CHAMBERS

16.3.1 General

Liquids may be dispersed as vapors or as aerosols. Solids may have been previously comminuted or it may be required to subdivide them in the process of dispersal. The choice of method to be employed should be based on the following criteria.

1. The dispersing technique must not produce any chemical change in the material.
2. The delivery rate must be constant during the experiment.
3. The rate of delivery should be readily measurable in order to provide a measure of the nominal concentration.
4. The rate of delivery should be readily adjustable to provide an adequately wide range of concentration.
5. The material must be dispersed in particles of desired size.

16.3.2 Techniques of Dispersal

The UCTL has had to test the toxicity of materials ranging in volatility from gases to metals. The physical state of a substance determines the method of dispersal to be used.

*Compounds Boiling Below 0 C.*⁶ These substances are usually available compressed in small steel or copper cylinders. A pressure-reducing valve is attached. A capillary or orifice flowmeter is then calibrated for the rate of flow of the gas by the liquid displacement method. A liquid in which the gas is insoluble is used in the flowmeter as well as in the pneumatic trough. The gas is delivered at the desired rate through the flowmeter directly into the gassing chamber. A nominal concentration, as a check on that derived from the rate of flow, is obtained by weighing the cylinder before and after each run. Unstable gases (e.g., ketene^{21a}) have been generated directly into the chamber.

Liquids Boiling Between 0 C and Room Temperature. These compounds may be dispersed as gases or, with proper cooling, as liquids. If they are to be treated as gases they are distilled into a glass ampoule. A calibrated flowmeter and a reducing valve are attached as described. The nominal concentration is obtained by determining the volume displaced during a run or by weighing.

It is usually more convenient to treat such compounds as liquids. With proper cooling, a solution may be made up. Any dispersing device for liquids which can be adequately chilled can then be used. Such devices are concentric and impinging atom-

izers, bubblers, and the small Benesh and constant-delivery atomizers.

Liquids Boiling Above Room Temperature. These may be dispersed by vaporizing or spraying. They are vaporized by passing nitrogen through a bubbler. The volatility of the material determines the size of bubbler and the degree of heating or cooling required. In general it is desirable to use as little heating as possible. Heat is supplied by a water bath at a temperature somewhat higher than that desired for the liquid in the bubbler.

Liquids may be atomized either undiluted or in solution. Solutions should not be dispersed from impinging atomizers since the solute and solvent are usually refluxed to different degrees with corresponding changes in concentration of the solution in the atomizer.

Solids Which Can Be Dissolved or Which Melt without Decomposition. A solution of a solid can be sprayed in the usual fashion. The volatility of the solvent is important. If too volatile it may evaporate sufficiently rapidly at the atomizer tip to produce clogging.

Agents which melt without decomposition can be dispersed from a direct or impinging atomizer immersed in a water or oil bath.

Solids Which Cannot Be Dissolved and Which Decompose When Melted. In most cases these materials must be ground to the desired particle size before dispersal. They can be dispersed from the dry duster (see Section 16.3.3 under "The Dispersal of Particulates").

Very fine aerosols of metals have been produced by means of an high-tension arc, using the metal as one of the electrodes.

16.3.3 Apparatus for Dispersal

DISPERSING BUBBLERS^{6,23}

A method of dispersing liquids with appreciable vapor pressures is to bubble a nonreactive gas through them. The output is controlled by varying the flow of the gas and the temperature of the water bath in which the bubbler is immersed. The gas passing through the liquid is broken up into small bubbles by passage through a sintered glass disk (coarse porosity) or a Folin bulb.

The type of bubbler used depends on the volatility of the toxic agent. Agents boiling below 50 C are kept in bubblers with stopcocks at both inlet and outlet to minimize the possibility of leakage when the bubbler and contents are being weighed at room tempera-

ture. Compounds with high boiling points are kept in bubblers with outlets large enough that the rapid flow of the gas mixture does not blow out material condensing in the outlet nozzle. When small amounts of agent are to be dispersed the bubbler should be kept small and light enough to be weighed on an analytical balance. (This also applies to atomizers.)

When substances are vaporized from bubblers it is desirable to keep the bath temperature as low as practical. This minimizes the decomposition of thermolabile agents. To get high concentrations in such cases increased gas flows are employed in large bubblers through which as much as 12 lpm of gas can be passed.

The use of bubblers in toxicity determinations is limited by the purity of the substance available and the amount of air (or nitrogen) which can be passed through them. If the toxic material is quite pure and stable the amount of substance volatilized per volume of gas passing through is quite constant. A very slight degree of impurity will, if the impurity is volatile, result in a changing output from the bubbler. As a result it is always necessary to make a series of preliminary runs to bring the output down to "constant volatility." Bubblers designed to hold 10 to 50 ml of toxic agent usually permit the passage of gas at a maximum flow rate of 2 lpm. The most constant operating conditions are obtained when the rate of gas flow through the bubbler is sufficiently slow to permit at least 95 per cent saturation of the gas with the vapor.

ATOMIZERS

An atomizer functions on the Bernoulli principle. A tube is positioned in the center of a jet of air. This tube is immersed in the liquid to be dispersed. The liquid is aspirated up the tube and sheared off the end. The size of droplets produced depends on the diameter of the tubing at its orifice, the viscosity of the liquids, and the rate of flow of air. The tube supplying liquid may be concentric with the jet of air or at right angles to it.

*Concentric Atomizers.*⁶ These are commonly made of glass. A capillary tube (A, Figure 2) is drawn down to a tip and bent at right angles and sealed into a bulb B. The tip of the capillary is adjusted so that it is precisely centered in the orifice O of the bulb. The annular space between the orifice O and the tip of the capillary is drawn to such dimensions that the desired delivery is obtained at air pressures of 5 to 20 psi.

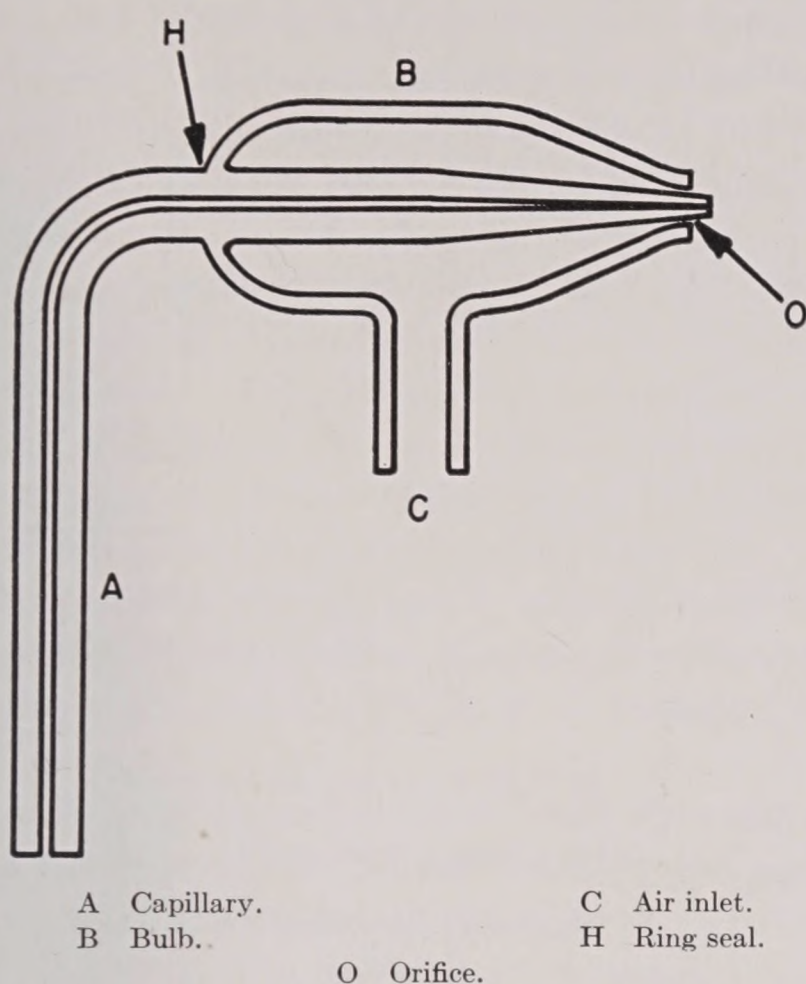


FIGURE 2. Concentric atomizer.

Maximum efficiency is obtained when the tip of the capillary either extends slightly beyond or is withdrawn slightly into the outer orifice. The adjustment is most readily made by heating the atomizer in the region of *H* (Figure 2).

The delivery rate and particle size are determined by the dimensions of the tip and the orifice.

A constant head device can be applied to the flask so that the delivery does not vary with the level of the fluid.

Concentric Atomizers — Constant Delivery Type. Concentric atomizers supplied with liquid solely by the Bernoulli effect are subject to variations in their delivery rate. The delivery rate decreases as the liquid level falls. Furthermore, the delivery rate is influenced by fluctuations in pressure of the gas driving the atomizer. At the UCTL certain atomizing units have been constructed which are provided with liquid by a constant-flow motor-driven pump. Two of these are called Benesh machines, after M. E. Benesh, Chief Engineer in charge of Research and Testing of the People's Gas, Light and Coke Company, who designed them.

1. *The small Benesh machine (Figure 3).*^{5,6} This machine consists of a chamber and an atomizer built into one compact unit. The all glass chamber has a volume of 18 l. It can hold 40 mice, 7 rats, or 7 guinea pigs. It is built with double walls between which a suction of 1½ inches of water is maintained. This prevents leakage of toxic material. To insert animals,

the whole chamber is raised by a rack and pinion. When the chamber is lowered, a gastight seal is maintained by rubber gaskets. A constant air flow of 180 lpm is maintained by a combination pump and meter driven by a synchronous motor. This is also geared to the mechanism for delivery of the toxic liquid, so that even if the motor should vary the same proportion of toxic agent to air would be maintained.

The agent is displaced from a buret by a rising column of mercury. It flows through stainless-steel tubing to a small stainless-steel concentric atomizer. The mercury column is connected through a U tube, omitted in Figure 3, to a brass cylinder filled with oil. A stainless-steel piston, 0.250 inch in diameter, is driven into the cylinder at a known constant rate. This drives oil into one leg of the U tube, and mercury out of the other. The piston is driven by a lead screw, connected through a change gear box to the synchronous motor. Three hundred and eighty-five gear changes are provided. A high-speed motor is belted to the lead screw for rapid return of the piston.

The main air stream is divided so that 158 lpm goes directly into the chamber while 22 lpm enters a compressor which feeds the atomizer. The spray from the atomizer enters a spiral evaporator which is provided with a flow of hot water of controlled temperature; the air stream of the atomizer may also be heated. Less volatile materials are condensed on and evaporated from the spiral coils.

An inverted mercury-water U tube provides an estimate of the nominal concentration and a check on the accuracy of displacement. In use, the mercury level is set at the zero mark in the first leg of the U tube. During the run, mercury is driven into this bulb, displacing water, which displaces mercury from the next bulb into the third bulb containing the solution to be displaced. At the end of the run the mercury in the first bulb is drained off back to the zero mark, and weighed. When the machine is free from leaks, better than 99 per cent recovery is obtained.

The Benesh machine is used as follows. Either the density of the agent is determined, or a solution of known density and concentration is made up. From this is calculated the revolutions per minute needed to give the desired concentration. The change gears are then set to give the correct rpm. It is usually possible to select a gear setting such that the rate of delivery is within 2 per cent of the amount desired. Revolution counters on the carriage are set to give the number of revolutions needed for a run of the

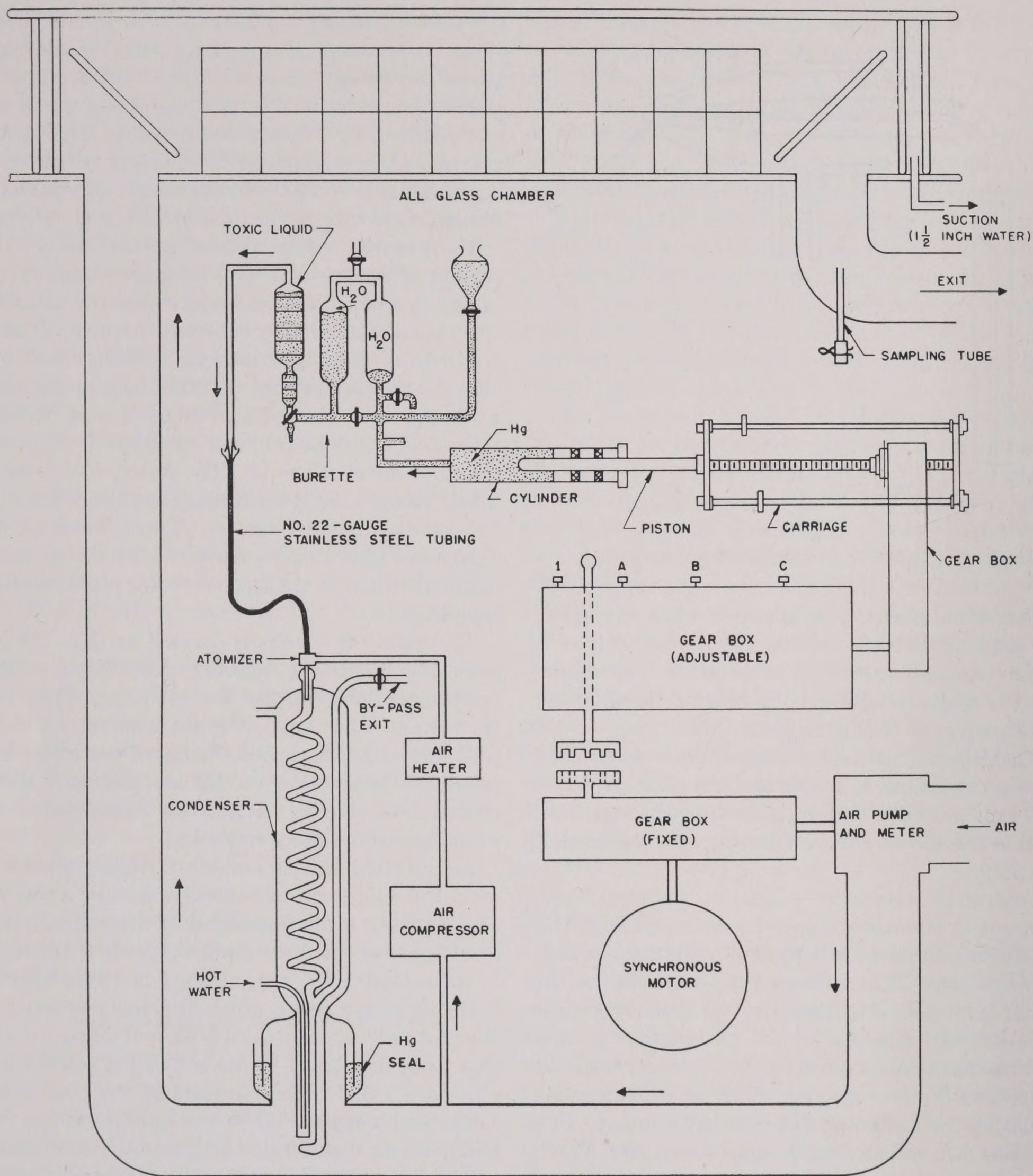


FIGURE 3. Small Benesh machine, diagrammatic.

desired time. The animals are placed on the chamber floor and the chamber lowered. The toxic agent is placed in the buret. The machine is placed in gear and started.

The exposure does not start until the first revolution counter is tripped. Prior to this, the agent is dispersed into the system, but exhausted before reach-

ing the chamber. When the first revolution counter is tripped solenoids are actuated which turn off the exhaust valve and open a stopcock to admit mercury into the measuring buret. The exposure continues until the second revolution counter is reached and thrown. This automatically disengages the motor and turns on the exhaust valve. The animals are then re-

moved. If a different concentration of the same agent is to be tested, it is only necessary to change the gear and revolution counter settings. It is possible to make as many as five 10-minute runs on the same solution within an hour.

The maximum concentration that can theoretically be attained with any substance is one-eighth of its equilibrium volatility at the temperature of the heating coils. Since the air flow through the coils is too rapid for saturation to take place, the actual concentration obtainable is somewhat less.

The machine should not be used with substances which attack mercury and stainless steel. In practice, materials which react slowly with mercury can be used.

2. *The large Benesh atomizer.* The atomizer on the small Benesh machine was permanently connected to one small chamber. It could not be used with aerosols, which came to form an increasingly important part of the work. The large Benesh machine was built to retain the advantages of the smaller machine in a somewhat more flexible form. The essential design was retained, but the following changes were made.

- a. The volume of the buret containing the toxic liquid was increased from 25 to 130 ml. This permitted longer runs or higher concentrations to be used.
- b. The oil-mercury system was somewhat cumbersome and prone to leakage. It was made necessary by the use of a brass cylinder. Changing the size of piston used was a major operation. In the large machine direct displacement of mercury was made possible by the use of an all-steel system.

Three concentric pistons, 1 inch, $\frac{1}{2}$ inch, and $\frac{1}{4}$ inch in diameter are used. The two larger pistons can be quickly locked down and used as cylinders for the next smaller size. The pistons and lead screw are mounted vertically on a section of channel which can be inverted for removing air bubbles from the cylinder.

The largest piston displaces mercury at the rate of 0.6435 ml per revolution of the lead screw; the other pistons displace in proportion to their areas. The stroke is about 10 inches, the maximum displacement about 130 ml.

- c. In place of the change-gear box on the small machine, three pairs of standard loose

change gears connected by idlers are used. These are changed by hand, with a wrench. Twenty sizes of change gears are available. By using the several gears and pistons available several thousand rates of displacement are theoretically possible, ranging from 0.008 to 7,000 ml/min. In practice both extremes are avoided, because of the inaccuracy of the first and the high pressures produced by the second.

The ease of changing gears in the small Benesh machine made it convenient to make up one solution and change concentrations by changing the rate of displacement. With the large Benesh atomizer it was frequently more convenient to leave the gears set at a certain ratio and make up different solutions for the desired concentrations.

- d. The atomizer, driven by a refrigerator compressor operating at 45 to 70 psi, sprays directly into the chamber rather than into a condenser. This makes it possible to use aerosols. Solutions of agents of low volatility, such as glycerine, may be used to produce aerosols of a size determined by the concentration of the solution. Thus 0.1 per cent solution gives clouds of mass median diameter [MMD] 0.3μ and 5 per cent gives an MMD of 4.0μ from droplets initially 7μ .
- e. This atomizer has none of the automatic controls used on the small Benesh machine. Return of the pistons is made by a hand crank geared to the lead screw.

This atomizer cannot be used with materials with low boiling points, inasmuch as no provisions were made for cooling the storage buret. It should not be used with materials which react with mercury or stainless steel.

3. *The small, constant-flow atomizer.*²¹ⁱ Many of the features of the Benesh atomizers can be retained in a very simple apparatus.

Standard, all-glass syringes are used as pistons and cylinders. These are connected to a small glass concentric atomizer. Connection to the atomizer may be made by an all-glass system, but a short piece of rubber tubing is preferred to prevent breakage. Syringes ranging from 1 to 100 ml may be used. They are held in two metal brackets by rubber collars.

The lead screw is geared by bevel gears to a 1/150 hp Bodine synchronous motor geared down to

3 rpm. No provisions for changing the gear ratio are made, and changes in concentration must be made by changing the syringe size and the concentration of solution. The machine is made to give 18 minutes of running, which allows for equilibration time and a 10-minute exposure. The machine will deliver from 0.044 to 1.74 ml/min with the various syringes. The piston is returned by reversing the motor and running the machine backwards. The syringes can be cooled by laying rubber bags filled with ice water across them. This cooling is adequate for a 75 per cent solution of hydrogen cyanide in ethanol. This machine is quite satisfactory. Nominal concentrations can be estimated from the change in reading on the syringe. It can be set up on almost any chamber and used with almost any agent. Slightly more complex design would provide changeable gear ratios and a quick return device.

4. *Modified Binks atomizer.*²¹¹ For work with the wind tunnel an atomizer was needed that would give an estimate of the amount of agent delivered at any time during a run. A commercial spray nozzle was modified for this purpose. The nozzle was a Binks #174 humidifier nozzle obtained from the Binks Mfg. Company of Chicago. The body of this is a bronze casting which contains a needle valve concentric to a conical air passage. An indicator arm fitted with a hairline was attached to the handle of the needle valve. A 360-degree protractor dial was fitted to the body of the nozzle. With these, precise and reproducible settings of the needle valve could be made. The fluid feed inlet was fitted with a 25-ml buret cemented into a brass sleeve threaded to fit the body of the valve. Compressed air at 10–25 psi was supplied through a corrugated hose fitting threaded into the lower inlet. At 15 psi and with a fluid feed of 2.0 ml/min, a cloud of MMD 8.0 μ was obtained. Finer sprays were obtained with higher air pressures and slower feeds.

The principal advantage of this atomizer is the direct reading of the amount of solution delivered during a run. The output tends to vary somewhat with the hydrostatic head in the system. The atomizer cannot be used with substances which attack brass.

*Impinging Atomizers.*⁶ In these atomizers the jet of spray from the atomizer strikes a baffle plate. Larger particles stick to the wall and run back to the liquid reservoir, whereas smaller ones remain airborne and are swept out of the chamber, either by the air blast from the atomizer itself or by an auxiliary air supply.

Theoretically these devices could use either a concentric or a right-angle atomizing unit. To save space the right-angle unit is commonly used. This unit must be adjusted before sealing into its container. The size of particles obtained (and inversely the output of the atomizer) is largely determined by the distance of the jet from the baffle plate. The wall of the container may be used as a baffle, or a small plate may be fitted in front of the orifice.

1. *Multiple-jet impinging atomizers.*²¹ⁱ These impinging atomizers work at a very low efficiency. Perhaps 5 per cent of the output of the atomizing unit passes out of the nozzle as aerosol. This results in a quite low delivery. It was necessary to develop an atomizer which would set up larger amounts of material as an aerosol for use in the wind tunnel. Impinging atomizers of partially metal construction were developed. The atomizing unit consists essentially of a hollow brass tube, about an inch in diameter, with the lower end plugged and the upper end connected to the air line. Near the lower end #55 holes are spaced equally around the circumference. A small brass tube, with the upper end machined, is soldered to the body of the cylinder at right angles to the axis of each hole. The lower end of this tube dips into the liquid to be dispersed; the upper end is centered in the jet of air from the hole. The most successful of the atomizers has eight of these jets. A shield in the form of a truncated cone is soldered base down around the units to form a baffle. This does not greatly affect particle size but it facilitates return of fluid to the bottom of the bowl.

The vessel in which these are placed consists of a 1-l Florence flask to the neck of which is sealed a side arm of the same diameter. The shape and diameter of the side arm determines the particle size. This side arm is fitted with a trap which returns liquid to the reservoir. A bulge on the bottom of the flask provides for efficient scavenging of small amounts of liquid. The diameter of all tubing through which the aerosol passes is kept as large as possible.

The eight-jet atomizer, operated with 5 cfm of air at 20 lb pressure delivers from 1.5 to 2.3 g/min with agents of low volatility. These atomizers can produce clouds of MMD from 2.0 to 3.5 μ .

These atomizers overcome the main drawback of impinging atomizers, i.e., low delivery. Impinging atomizers cannot be used with binary systems, as they fractionate them, the more volatile component distilling over. Impinging atomizers tend to give a flat and fairly linear curve for output versus

pressure, which makes fine adjustments in output practical.

THE DISPERSAL OF PARTICULATES

Certain of the atomizers used above may be used for the dispersal of particulates, as solutions or molten solids, as well as for vapors. There are in addition several methods peculiarly adapted to the dispersal of particulates.

The Dry Dusting Atomizer.^{19,21c,27h} It became necessary to test the toxicity of dry dusts in comparison with that of atomized droplets of solutions. The "dry-duster" was developed for this purpose. It is essentially an atomizer for dispersing dry powders. The body of this atomizer is a straight tube, 25 mm in diameter. It is separable in the middle by a ground-glass joint, for ease in filling. A glass nipple is sealed to the lower end. A sintered glass disk (40–60 mesh) is sealed across the bottom of the lower member, just above the constriction. The powder is placed on this sintered disk. A side arm, constricted distally, is sealed to the upper member. A tube side of smaller diameter is ring-sealed through the opposite wall to extend concentrically into the side arm.

In operation this device is charged with powder, assembled, and placed in a flexibly mounted clamp. A clamp attaches the assembly to an eccentric mounted on the shaft of a small electric motor. The vigorous agitation so provided tends to prevent channeling, and to ensure a uniform rate of dispersion. The two concentric tubes sealed to the upper portion constitute an atomizer. The Venturi vacuum produced by passage of compressed air through the inner member draws a current of room air through the sintered disk. This current draws the particles up to the atomizer and into the chamber. Much closer regulation of the output is possible if instead of relying on the vacuum, a slow current (1 lpm) of dry nitrogen is passed through the lower inlet.

A good estimate of the nominal concentration is provided by the weight loss of this duster. The apparatus disperses particles at approximately their original size. The shearing action of the air blast shatters aggregates to a certain extent.

Fractionating Devices. As it is difficult to obtain clouds of a desired particle size, a fractionating device is sometimes introduced between the atomizer and the exposure chamber. Two forms of fractionators have been used.

1. *Fractionating tower.* This device makes use of the fact that the mass and volume of a particle de-

termine its rate of settling. This relation is formulated in Stokes' law. By passing a current of air up a vertically mounted tube those particles which fall at a velocity greater than that of the air current will settle out; smaller particles with slower rates of fall will be swept up the tube. Such a tower may be used to reject either small or large particles, depending on whether the outlet for desired particles is placed at the top or the bottom of the tube.

A tower of this sort was used for work with one particulate^{21b} to exclude all particles above 5 μ in diameter. One has been used for work with another agent dispersed from an impinging atomizer in the molten state.^{21h} In this tower particles below 75 μ were sucked upward and rejected, while the larger particles were allowed to fall downward into a small wind tunnel.

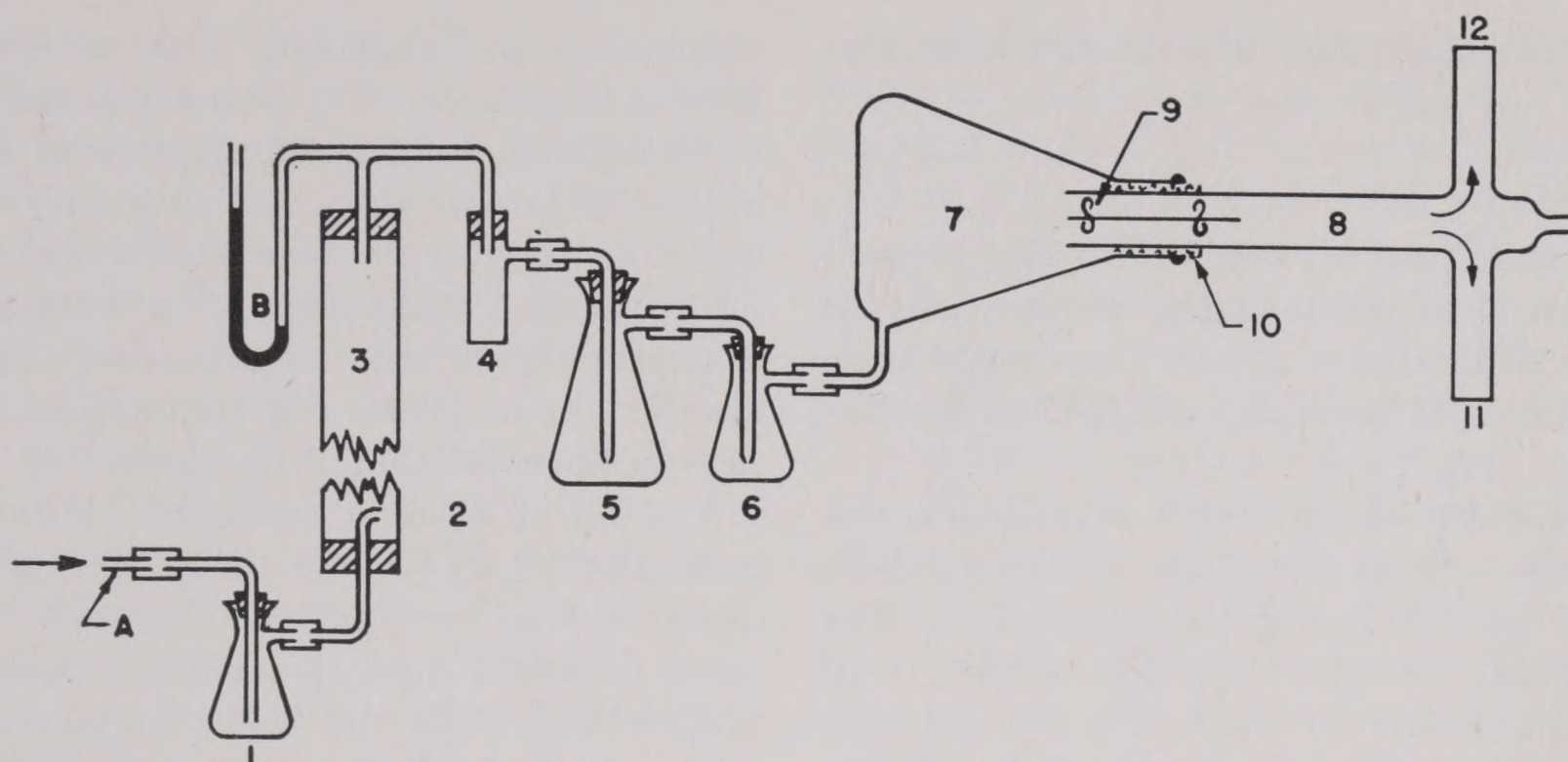
2. *Rotating macro-impinger.*²¹ⁱ One of the principles widely used in analytical instruments for aerosols has been that of impingement. A jet of aerosol-laden air is driven at high velocity against a surface. The larger the particle, the better its chance of sticking.

An attempt was made to use this method on a larger scale to reduce the mass median particle diameter of a dry aerosol (see Chapter 12). This was done by impacting the dispersed agent at a high velocity against a moving kymograph drum which had been coated with vaseline. A moving surface was used for impaction to prevent overloading. The smaller particles which did not stick to the drum were passed into an exposure chamber.

This equipment was able to reduce the MMD only from 6.3 to 3.8 μ . It was somewhat bulky and its use was abandoned. However, this method of reducing the MMD has certain inherent possibilities.

3. *Serial macro-impingers.*²¹ⁱ Large impingers (see Figure 4) operated in series have been successfully used to reduce the MMD of a NaHCO_3 cloud below 1.0 μ . Filtration flasks lined with vaseline are used, with a central tube extending down from the top. The size fraction taken out is regulated by adjusting the position of this tube and varying the air flow. This method is less cumbersome and more effective than the rotating impinger.

Electrical Atomization.^{21a} This method of dispersal is peculiarly well adapted to the dispersal as aerosols of metals and other conductive, heat-stable materials which are not obtainable in a finely powdered form. The material to be dispersed is used as an electrode for a high-voltage arc. If the material is to be dis-



- A Critical pressure orifice from compressed air line at 15 psi (delivers 38 lpm).
 B Manometer (maintained at 3 psi).
 1 Flask holding powder to be dispersed.
 2 Air jet (directed to side to facilitate mixing).
 3 Settling column (130 cm high, 5 cm in diameter).
 4 Vaseline-coated impinger.

- 5 Macro-impinger (1-liter filter flask containing Vaseline and oil mixture).
 6 Macro-impinger (500-ml flask as above).
 7 Mixing flask (2-liter).
 8 Manifold.
 9 Stationary fan blades of opposing rotation.
 10 Cotton plug (permits escape of excess air).

11, 12 Exits to mask and to sampler.

FIGURE 4. Dry cloud apparatus.

persed as an oxide, the arcing is carried on in an atmosphere of oxygen; otherwise helium or hydrogen may be used. The aerosol is mixed with dry air and led into a small chamber; regulation of the concentration is achieved by varying the amount of diluent air.

Aerosols obtained by this method are extremely fine, somewhat less than 0.3μ in diameter. The output of the arc is quite constant, and regulation of the concentration is made by regulating the air flow.

16.4 SAMPLING EQUIPMENT

It is usually necessary to know not only the concentration of agent put up in a chamber, referred to as the "nominal" concentration, but also to know the concentration actually existing in the chamber. This concentration is determined by chemical or physical methods and referred to as the "analytical" concentration. With vapors, it is only necessary to know how much of the material is dispersed in a certain volume of air. With particulates, in addition to this information, it is necessary to know something about the size of the individual particles.

16.4.1 Equipment for the Sampling of Vapors

Most of the apparatus for determining the concentration of vapors in gassing chambers is of common

use in the study of air pollution.³⁴ UCTL practices are as follows.

1. Withdrawing of the gas sample is effected by either a water aspirator or an electrically driven pump.

2. Measurement of the volume withdrawn is usually made by a wet test meter. This meter is calibrated by the positive displacement of a known volume of air through it. Familiarity with methods used in field trials led to the use of critical pressure orifices for regulating sampling rates.

3. The type of absorbing bubbler most commonly used in this laboratory is made of glass. A coarse sintered disk is used to break up the gas into bubbles.

An investigation was made of the efficiency of three types of bubblers,²¹ the sintered disk type,⁶ the Bushnell type, which has a plain inlet tube extending about 5 cm into the absorbing liquid, and the Edgewood type, which is filled with glass beads. The absorption of H was studied, and a Northrup titrimeter used to measure the slippage. The sintered disk type was found to be the most efficient. The following conclusions governing the use of bubblers were drawn from this study and coincide with others independently obtained.¹⁵

1. The absorbing solvent should have a low vapor pressure.

2. If possible the solvent should react with the absorbate to give a nonvolatile compound.

3. The absorbent should dissolve water vapor if the air has an appreciable humidity.

4. A solvent which foams considerably is to be preferred to a nonfoaming solvent, other factors being equal.

5. Low temperatures are conducive to better absorption.

6. The flow rate of the gas should be kept as low as possible.

7. The kind of bubbler is of less importance than has usually been assumed.

Two devices for the analysis of vapors have been developed at the UCTL. They are described below.

Low-Resistance Absorber.^{27c} Investigations of the hydrogen cyanide content of expired air ("precision" gassing) required a bubbler that would combine low resistance, small volume of absorbent, and small dead space with high efficiency at intermittently high flow rates. It was necessary to design a new type of bubbler to meet these specifications.

This absorber consists essentially of a pair of concentric glass tubes. The outer is 37 cm long and 2.2 cm inside diameter. Inside, it is a tube with both ends closed, 33 cm long and of such a size as to leave a 1-mm annular space between it and the outer tube as an air passage. The outer tube is fitted at its ends with male ball joints so that it can be freely and continuously rotated about its long axis (Figure 5). It is

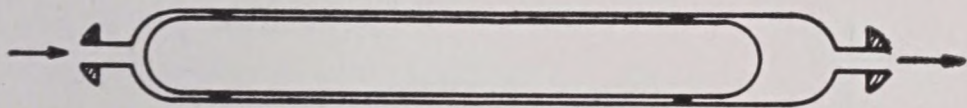


FIGURE 5. Low-resistance absorber.

rotated by two micarta pulleys, 2 inches in diameter, bored out to fit the outer tube, and cemented to it. These pulleys rest on the rollers of a small ball mill (Fisher Minimill). The whole assembly is mounted on two rods attached to the sides of the ball mill. Two brackets hold the corresponding female ball joints flexibly. The absorber is held down against the rollers by helical springs attached to slip rings.

In use 8 ml of absorbent are poured into the absorber. This is more than enough to wet all exposed surfaces when the absorber is rotated. Thereby the absorbing surface is continually being renewed. Tests have shown resistance to be very low, about 1 cm of water at an air flow of 30 lpm. When 3 per cent NaOH in ethanol is used as an absorbent, the absorption of hydrogen cyanide is 100 per cent from air containing 2.4 mg/l and flowing at 7 lpm. It is 95 per

cent when the flow rate is 30 lpm. Only 30 ml of wash liquid are needed, as compared to 500 ml needed for a bead bubbler of somewhat lower efficiency. The absorber and its motive power form a fairly compact unit.

*An Electronic Interval Timer for the Northrup Titrimeter.*¹² The Northrup titrimeter is an electrochemical analytical instrument⁹ for the quantitative determination of the airborne concentrations of chemical warfare agents (see Chapter 36). A sample of contaminated air is drawn in at a constant rate. At intervals it is titrated with a dilute bromine solution. The titration is carried on in one half of an Ag/AgNO₃//Br₂/Br⁻ cell, with a platinum indifferent electrode. When oxidation is complete, an excess of bromine creates an electrical potential, which is recorded on a galvanometer. The amount of bromine solution needed is determined by the time required for it to flow from a constant-head buret.

This instrument is made in two forms. In the simpler field model the bromine solution is run in by the operator, who shuts off the flow when the galvanometer shows a positive reading. In the automatic model the galvanometer mirror reflects a beam of light on a photocell when the titration is complete. The photocell actuates relays which shut off the buret and start another sampling period. The length of sampling period is controlled by fixed cams, which give a choice of four periodicities; from 1 minute sampling and 2 titrating to 50 minutes sampling and 10 titrating. Between the end of one titration and the start of the next sampling period the cell is kept in equilibrium — the agent sampled during this period is balanced by intermittent addition of bromine. The opening and closing of the bromine buret is recorded by a relay-actuated marking pen writing on a paper record wound around a kymograph drum.

In this form the automatic model was incapable of accomplishing some of the determinations that were desired at UCTL. In particular, the shortest time interval available (cycle repeated every 3 minutes) was too long for showing variations in concentration occurring at a frequency greater than that, whereas the provision for 1-hour sampling periods was unnecessary. The cyclic rate could have been increased by cutting another cam. However, it was desirable to eliminate the time lost between the end of one titration period and the start of the next sampling period which results from the use of the cam timing mechanism.

An electronic method was adopted. The sampling

time interval is governed by the time required to discharge a condenser of high capacity through a high resistance. The resistance was controlled by a potentiometer; changing this setting varied the time of discharge, and hence the sampling rate. The sizes of the elements used were such as to give continuous variation between 0.25 and 5.8 minutes; a longer sampling period proved unnecessary but use of larger condensers would provide it. At the end of the sampling period titration starts and continues until all the agent collected during the pretitration period plus that collected during the titration period is titrated. Thereupon the titration stops and essentially instantaneously a new cycle begins.

This addition to the laboratory model Northrup titrimeter has the following advantages.

1. Continuous variation in sampling times is available merely by turning a knob.
2. As soon as one titration period has been completed, a new sampling period begins.
3. By using short time intervals the concentration of agent in the absorption cell is kept very low at all times, thus reducing the loss of material by slippage.
4. The original instrument is now adapted for use in determining concentration changes in gassing chambers over short periods of time.

16.4.2 Equipment for the Sampling of Particulates⁶

Filters. One of the simplest ways to determine the concentration of a smoke is to draw a measured sample of air through an efficient filter and determine its gain in weight. At UCTL much early work with smokes was done with cotton-asbestos mats (40 per cent cotton — 60 per cent asbestos) 1 to 2 mm thick, pressed into perforated or sintered glass filtering funnels (25 mm in diameter). Suction of from 0.6 to 2.0 inches of mercury was needed to pull 3 to 4 lpm through these.

Work on certain types of aerosols (see Chapter 12) introduced several new requirements for a filter.^{21d} Since the determinations involved a micro procedure, it was necessary that the filter material have a low blank (less than 20 μg). The filter chosen should not be clogged by as much as 10 mg of a standard preparation and should offer low resistance to air flow. Several filter papers were tried before one made from cellulose acetate was found to be satisfactory. This paper contained no material simulating the material determined in the analytical procedures. Insoluble material could be completely floated off, and the batt

could be completely dissolved in a suitable organic solvent, leaving the particles unaffected and ready for counting.

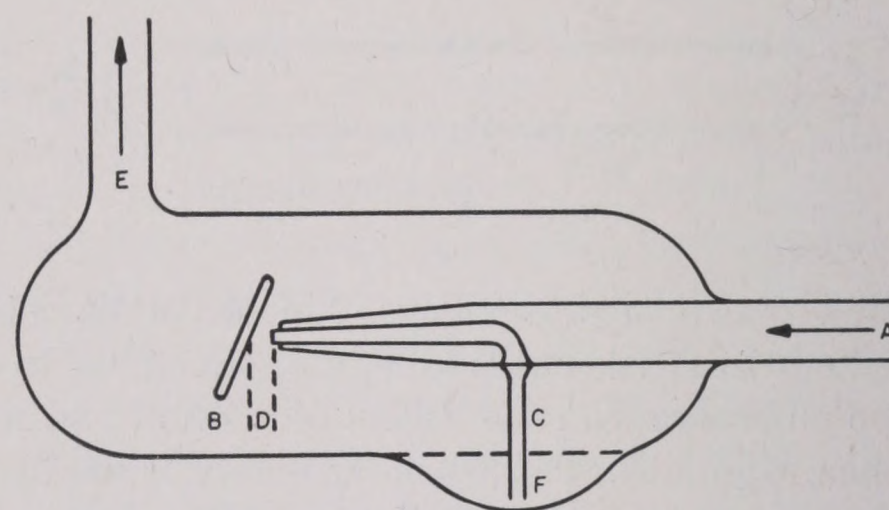
In use, both in the laboratory and in the field disks of the paper are stamped out. They are held in brass holders in which they are backed with a wire screen.

Precipitators. Particles have been removed from the air by direct precipitation. A Watson⁴⁰ thermal precipitator has been constructed and used. In this instrument the air passes over a Nichrome wire kept at 100 C. Smokes are precipitated on cover slips backed by brass blocks.

Electrostatic precipitation has also been used in a small Cottrel-type precipitator.³³ This is essentially a long glass cylinder. Copper screening is wrapped around the outside, and attached to one terminal of a 15,000-volt transformer. A wire in the tube at its long axis forms the other pole.

Impinging Devices. An impinging device is one in which the smoke-laden air is drawn or driven at high velocity against a prepared surface. The particles may be trapped on the baffle plate or absorbed by some liquid medium. Impinging devices may be used either to collect all of the particles in one stage or to fractionate the particles by using jets of various speeds.

1. *The atomizing impinger.*^{21c} This unit (see Figure 6) consists of a concentric atomizer mounted in-



- A Inlet tube.
- B Baffle (supported by rod from A, not shown).
- C Capillary.
- D Distance between capillary and baffle.
- E Exit to vacuum.
- F Fluid to be atomized.

FIGURE 6. Atomizing impinger.

side of a glass vessel in such fashion that its jet strikes a baffle plate and drains down to a sump from which it is re-atomized. Dust particles in the incoming air ring strike the baffle plate and are trapped.

A straight tube is ring-sealed concentrically through the top of a side arm test tube. The inner tube is constricted at its inner end to give a jet. A

smaller tube is ring-sealed through the wall of this inner tube in such a fashion that its outer end extends at right angles, and its inner end is concentric to and extends through the constricted portion, forming a concentric atomizer. This outer end dips down into a bulge on the outer tube which acts as a sump. The apparatus is mounted horizontally. A glass arm supports a baffle plate which is carefully positioned in front of the jet. If this plate is too close to the orifice the atomizer will not function; if too far away the unit will act as an atomizer and not as an impinger. A source of vacuum is attached to the side arm, and the inlet tube connected to the chamber.

This pattern has proved 90 per cent efficient in the collection of clouds which had been allowed to settle for 30–40 minutes and contained particles with an MMD of $6\ \mu$. A practical advantage of the apparatus is that the collecting volume is small and, consequently, small amounts of toxic agent are not diluted too much for injection into animals.

The Cascade Impactor. The construction, method of use, experimental results, and theoretical principles of the cascade impactor are fully described by K. R. May.³⁶ Experiments at the UCTL have emphasized the desirability of the instrument with dry particles and have employed slightly different calculation procedures, such as the substitution of the MMD on each plate for the effective drop size [EDS] used by Porton. With dry clouds a total sample of about 0.350 mg represents the maximum which can be obtained without overloading if the cloud is distributed on all four slides. With dry powders the presence of aggregates in the airborne cloud complicates the calculation of the MMD. These aggregates frequently break up upon impaction so that they cannot be measured microscopically. Since the density of an aggregate is lower than that for unitary particles, the aggregate is impacted along with unitary particles of smaller size. This property leads to the recommendation (Chapter 15) that particulates should be assessed in terms of impactibility rather than in terms of diameter.

1. *A device for increasing the load on cascade impactor slides.*^{21g} The amount of a particulate impacted upon slides No. 3 and No. 4 of the cascade impactor must be kept very small to prevent overloading. The quantity obtained on one streak is barely within the limits of the available analytical methods. To allow collection of a larger sample, a method of moving the slides at intervals during sampling was devised so that it is now possible to obtain eight streaks on

slide No. 4 and four streaks on slide No. 3 during one sampling period. A heavier cap is screwed on to the appropriate tubes of the cascade. In the center of this cap a hole is drilled and tapped for a $\frac{1}{4}$ -inch bolt. The bolt is passed through this cap. The inner end bears on the slide. This bolt is turned by hand at intervals during the run to move the slide. The distance it is moved is determined by the number of rotations of the screw.

2. *A modified cascade impactor for use with small particulates.*^{27d} The cascade impactor was originally designed to handle the range of drop sizes set up by munitions in the field. In work with nasal filtration of small droplets it was necessary to work in a range of much smaller sizes. The larger drops in this range were of about the smallest size that the standard cascade impactor would handle at 17.5 lpm. The standard cascade with critical orifice impinger and filter backing would trap the particles in this range but would not fractionate them.

A modified cascade was constructed which fractionated the drops which slipped past slide No. 4 and were caught by the impinger. The standard cascade has a rate of flow through its jets of 5, 30, 50, and 80 mph, with the impinger giving 700 mph. The modified cascade has jet velocities of 56, 86, 177, and 700 mph, the last slit being a critical orifice. (A critical orifice gives a speed of flow equal to the speed of sound — approximately 700 mph.) It will be noted that the first two jets of this cascade correspond, roughly, to the last two jets of the standard cascade, while the last two correspond to the impinger, as used with the standard instrument, and an intermediate value. This modified impactor has proved capable of efficiently fractionating a cloud which slips past the standard instrument. This modified instrument requires a backing filter to collect all material.

The present experimental model is blown of glass. The four separate sections fit together with rubber stoppers. The slides are held in place between indentations in the walls. Wall losses can be easily detected by inspection.

Particle Fractionators. Drop traps, chemical noses. In an attempt to simulate the characteristics of nasal passages with respect to particulates, various devices have been made to fractionate the cloud into a lung fraction and a nose fraction.

Glass tubing in the form of Z's and S's such as were used by British workers for liquid droplets were coated with a sticky film of alkyd resin in an effort to fractionate dry particulates.^{28–30} Better results

were obtained with selectors which were essentially the first stage of a cascade impactor backed by a filter.^{21f}

Wires for Sampling Particulates. The use of slides, tubes, and wires of different dimensions for the determination of particle size and cloud concentration has been described in detail.^{27a,b,e}

16.5 METHODS FOR "PRECISION GASSING"

In the usual gassing procedure no account is taken of the effect of the toxic agent on the respiratory volume or rate. Consequently, there is no means of determining the inhaled LD_{50} from the LC_{50} . Some species, especially rabbits, hold their breath to agents which are apparently undetected by other species. Methods which take account of the actual respiration during exposure have been termed "precision gassing" methods. A tracheal cannula and Douglas bag were employed in studying the effects of phosgene on the respiratory pattern of dogs.^{21e} In some instances the respiration was modified by the use of CO_2 .^{21f}

For the early investigations on the effects of hydrogen cyanide, the animal was mounted in a body plethysmograph attached to a Brodie bellows.^{27j}

More precise methods were later developed^{27b,c,f} in which a mask was fitted to the animal. A valve with minimal dead space was used and a special low-resistance absorber constructed.

16.5.1 Equipment

The Mask. The first mask tried was made of vinylite sheeting, shaped in a truncated cone. The apex of this was cemented to the male half of a 16/22 standard taper joint. This was used with dogs. The animal's snout was taped shut, and the cone bound over it so that the apex of the cone was in contact with the nostrils.

When this facepiece was applied in the usual manner, an average leakage of 17.5 per cent was found. When it was applied very tightly, the leak was reduced to an average of 4.4 per cent. In order to achieve this low leakage the facepiece had to be applied with sufficient pressure to embarrass respiration seriously.

This facepiece subsequently was replaced by a pair of nasal tubes beveled at one end to facilitate insertion. These tubes ($1\frac{1}{4}$ inch long, and 4 mm inside diameter) were attached by paragon rubber tubing

to the diverging arms of a glass Y tube sealed onto the end of a 15/20 male standard taper glass joint. The tube ends were closely approximated to the Y arms so as to expose as little rubber surface as possible to the agent. After gassing no adsorbed agent could be detected on the inside of the nosepiece.

It was possible to handle animals so intubated with local anesthesia alone (cocaine or butyn) but this was not entirely satisfactory. Therefore intubation was carried out on lightly anesthetized animals (for dogs, 20 mg/kg of Nembutal intravenously) after a swab of 1 per cent cocaine or butyn had been applied to the nostrils to prevent sneezing. The mouth was closed with elastic bandage and the tubes were then inserted and fixed with additional tape. No leakage could be detected in 11 of 12 animals tested, and the other had only 1.9 per cent leakage.

The Valve System. The first valve used was a copy of the all glass valve designed by Weston and Tobias.²⁵ The body of this valve was made from two female and one male 16/22 joints sealed together in a T. The long arm was made from a male and female joint, and the side arm from a female joint. In use the long arm is mounted vertically with the male joint at top. The valves proper are composed of glass disks ground flat, of such diameter as to fit inside the female joint. The end of the male joint is ground flat and serves as a valve seat. The disks are held down in place by their own weight. The assembly of male joint and disk is inserted in a female joint which keeps the disk in line. Such a valve will pass air in the direction away from the male joint.

In use, the dog's snout is connected to the female joint on the side arm. The lower valve is connected to a gassing chamber, and the upper, through a suitable absorber, to a spirometer. On inspiration the lower valve opens and permits contaminated air to enter. On expiration, the lower valve closes, and the upper valve opens and passes air to the absorber.

It is desirable to minimize the dead space as much as possible. The use of glass imposes limits on the reduction which can be made. In addition the convulsions of exposed animals place great strain on the valve.

It was possible to machine a valve from brass which would have less dead space. The valve is of the same general design, except that both the body of the valve and the flaps are made from brass. The valve has only 13 ml of dead space as compared to 25 ml in the glass valve, and is practically unbreakable. It was found that while untreated brass ab-

sorbed appreciable quantities of cyanide, brass "blued" by immersion in a hot solution of As_2O_3 in HCl did not react with cyanide.

The Absorber. The use of an absorber to collect gases from air, as expired, imposes certain peculiar requirements. It must be efficient at intermittently high flows (as much as 30 lpm), it must have low resistance (conventional absorbers have a resistance of several inches of mercury under these conditions), and it must be possible to rinse it out with a small volume of liquid, since very small quantities of agent are present in the expired air.

The first absorber used was the Edgewood low-resistance, glass-bead type.²⁵ This absorber is filled with fluid and drained just before use. Thus, the gas passes over the surface film and not through liquid. However about 500 ml of wash liquid was needed to transfer all the absorbed agent to the titration vessel. With the very small amounts of material present, this large volume of solution led to appreciable titration error. The UCTL low-resistance absorber^{27c} described (Section 16.4.1) proved more satisfactory.

16.5.2 Determination of Inhalation Toxicity of Particulates

As described in Chapter 15, the effect of particle size on physiological action is appreciable. In order to determine whether an inhaled particle was trapped in the nose or in the lung or whether it was exhaled again, various methods were devised similar in principle to those employed in "precision gassing" experiments with vapors but modified for the assessment of particles.

1. One procedure for use with animals involved exposure to toxic clouds of controlled particle size. It was used with agents which are highly toxic in the lung but of negligible toxicity in the nose. From these experiments the relation of particle size to toxicity in mice, rats, and rabbits was determined (Chapter 15).

2. Correlated with these experiments were methods for the determination of toxicity by intrapulmonary instillation of solutions of the toxic agent.^{27e}

In general the trachea of an anesthetized animal is cannulated, and the solution instilled into the trachea through a tube which fits inside the cannula. The method has previously been used for rats.³⁵ A small catheter is used. The neck is transilluminated with a Spencer microscope lamp to aid observation of the trachea.

For mice this method was modified as follows. A

cannula which fits snugly into the trachea of a 20-g mouse is made by rounding off the beveled tip of a 1½-inch 18-gauge needle. A 1½-inch handle is attached to the hub. The solution to be instilled is contained in a ¼-ml tuberculin syringe tipped with a 25-gauge needle. The mouse is etherized and tied on its back. The mouth is held open and the tongue held against the mandible with a pair of blunt forceps. The throat is transilluminated and the larynx is visible as a bright spot opening and closing with the respiratory movements. The cannula is inserted into the trachea through the larynx. When the cannula is situated correctly, it is possible to cause a pulsation of the chest by blowing gently into the cannula. The 25-gauge needle is then inserted into the cannula, and the solution instilled. When in position, the tip of the 25-gauge needle should be flush with the tip of the 18-gauge cannula. If the cannula is in the right position, the breathing becomes labored upon insertion of the 25-gauge needle, and returns to normal when the needle is removed.

16.5.3 Nasal Filtration and Lung Retention^{21k, l, 27f-i}

The mensuration of these factors involves setting up a particulate cloud and determining its particle size with cascade impactors before and after passage through portions of the human respiratory system. Obviously these tests could be done only with non-toxic materials. Either nonhygroscopic or hydrophilic aerosols may be used; dyed corn oil and calcium phosphate were used for the former, and NaHCO_3 for the latter (see Chapter 15). The basic techniques involved in these determinations are the same; differences will be discussed under the subheadings.

Setting up the Particulate Cloud. In the first work corn oil (Mazola) dyed with Sudan Red was sprayed from the large Benesh atomizer. When NaHCO_3 was used, it was dispersed from a 250-ml Erlenmeyer flask with a two-hole stopper. A glass tube drawn out to a fine tip extended through the stopper to near the bottom of the flask. When compressed air was forced through this jet, a cloud of NaHCO_3 dust emerged from a tube in the other hole of the stopper. This cloud was directed into a 12-l bottle, and the large particles allowed to settle out for 5–10 seconds. The particles remaining airborne were then drawn into the common entrance of the Y tube. It was found early in the experiments that the cloud dispersed as described was quite heterogeneous. Though

passing through a common tube, samples drawn simultaneously from the two arms of the Y tube showed markedly different distributions on the impactor slides. This was remedied by placing two small, oppositely oriented, metal propellers in the common tube. This stirred the passing air sufficiently to make the cloud uniform.

The same setup was used for calcium phosphate smokes. Further fractionation was provided by the use of serial macro-impingers, lined with Vaseline (see Figure 4).

1. *Dry cloud apparatus.* The dry cloud of NaHCO_3 was set up by the apparatus shown in Figure 4. Fifteen pounds pressure in excess of atmospheric applied to a critical pressure orifice for 18 lpm (at atmospheric pressure) gives a flow of about 38 lpm, sufficient to supply the lung and the control without dilution by unfiltered room air. The material enters the settling column. The agitation here is adequate to maintain the cloud at nearly the same concentration.

The pressure in the manometer was about 3 psi, practically all of it being due to impinger No. 6. The jet in impinger No. 4 was simply a large glass tube. In No. 5, the end of the tube was somewhat flattened, whereas in No. 6 the orifice in the end of the tube was about 1x5 mm. For smaller clouds a still smaller jet may be used, the pressure in the column being larger. To maintain the same flow rate only a slight shift in the initial pressure is required. If one starts with considerably higher initial pressures with a proportionately smaller orifice in No. 1, changes in pressure in the column may be ignored.

Between runs the impingers were warmed to resurface the bottoms of the flasks and the mixing flask 7 and tube 8 were blown clean. At the end of a run the last flask should not be too heavily coated.

Passage of the Cloud through a Portion of the Human Respiratory System. The experiments conducted were:

1. *Nasal filtration with corn oil.* The oil was sprayed from the Benesh atomizer into the 700-l chamber. This was operated at a flow of 300 lpm and acted as a settling chamber. A glass Y tube, 22 mm in diameter, led from the chamber. One branch of this Y led to a mask which fitted tightly over the nose and mouth of the subject. This mask was from a commercial dust respirator; an inflatable rubber tube formed a tight gasket with the subject's face. Protruding into the mask was a second exit tube about which the subject closed his mouth. The exit tube led through a cascade impactor, backed by an impinger, to the pump. The second branch

of the Y tube led to another impactor, also backed by an impinger and the pump. This impactor sampled the incoming cloud. The tubing between it and the fork of the Y tube was comparable in length and shape to the tubing which led to and from the mask. As far as could be controlled, the only difference between the two air streams was that one passed through the subject's nose and the other did not. The impingers backing the impactors were of such size as to be critical orifices, with a flow of 17 lpm. Each experiment was done in duplicate with the positions of the control cascade with its corresponding impinger, and the mask exit cascade with its impinger, reversed during the second experiment. This canceled out instrumental errors.

Sealed to the mask exit tube and preceding the cascade impactor was a small glass tube through which air could be drawn from or added to the system. This could be used to increase or decrease flow rate through the mask while maintaining the same flow through the cascade impactor. A similar tube preceded the control cascade. A second small opening in the tube which connected the mask to the cascade impactor led to a manometer which indicated the reduction in pressure caused by the resistance of the nose when air was flowing through it.

During each experiment, which lasted 30 seconds, the subject held his breath so that there was no appreciable passage of the aerosol into and out of his lungs. For the flow rate of 10 lpm, the 30-second run was repeated immediately in order to obtain a larger sample. The nasal resistance was found to be very low except in individuals whose nasal passages were congested or who were not sufficiently relaxed during the experiment. If a person is tense, his posterior nares may become constricted, with a marked increase in resistance. Subjects were used in the experiments only after they had had sufficient practice on the apparatus to allow a 10 lpm flow with a resistance of 0.3 inch water, 17 lpm with 0.5 inch, and 29 lpm with 1.0 inch or less. In the case of some subjects this low resistance could often be achieved only after inhalation of benzedrine, or the nasal instillation of neosynephrine solution.

2. *Nasal filtration with NaHCO_3 particles.* Commercial powdered NaHCO_3 was chosen as a nonirritating, nonaggregating powder containing particles of a size range from 1 to 15 μ (microns). The same assembly of mask, Y tube, impactors, critical pressure impingers, and pump was used as with the corn oil work. The impactor slides were covered with

alkyd resin, and the NaHCO_3 on them analyzed electrometrically.

The hygroscopicity of NaHCO_3 introduced several difficulties because of the moisture picked up by passage through the nose. It was necessary to oven-warm the impactor before use, and to warm the cloud from the nose by passing it through an 8-inch length of 15-mm tubing, electrically heated to give an emergent air stream of approximately 90 C. A duplicate heating device was used in the control air stream. The wetting and subsequent drying of the cloud passing through the nose compacted and rounded the particles in it. It was necessary to humidify the control cloud also. The humidifier was a metal tube (1 foot long, 1 inch inside diameter) lined with a water-soaked blotter, placed in a thermostated water bath. This humidifier was also an inefficient impactor, taking out about 20 per cent of the airborne material in the cloud going through it. A similar tube, lined with Vaseline, had to be placed in the path of the cloud to be transmitted through the nose. After these modifications quantitative agreement between the NaHCO_3 contents of the dry and humidified cloud could be obtained. With the use of cellulose acetate filters instead of the cascade impactors it was unnecessary to have the various driers.

3. *Retention of particles in human lungs.* In these experiments the subject's nose was plugged. Stop-cocks were placed in each of the two sampling tubes between the critical orifice and the filter.

During an experiment the subject inhaled for a fixed period, the beginning and end of which were indicated to him by an operator. During the inhalation period a sample of the incident cloud was drawn through filter A, by opening the corresponding stop-cock (the vacuum pump operated continuously). During exhalation, which was also for a fixed number of seconds, the exhaled cloud was drawn through filter B. The cycle was repeated 10–15 times, depending on the length of inhalation and exhalation periods.

Since the incident and exhaled clouds were sampled at the same rate and for equal periods, the material found in the exhaled cloud represented the unretained fraction of an inhaled quantity equal to that on the other filter.

The total volume breathed during an experiment was governed by the rate of withdrawal of the exhaled cloud from the mouth. The volume withdrawn during each period was considered to be the tidal air. The exhaled tidal air was, of course, constant from period to period since it was controlled by the sam-

pling instrument. The inhaled tidal air, however, varied from period to period depending on whether or not the subject inhaled a volume which exactly compensated for the amount withdrawn during the previous exhalation. Over a number of cycles, of course, the average volume inhaled had to be equal to the volume exhaled.

This method has been studied with smokes of NaHCO_3 and calcium phosphate. Since calcium phosphate is nonhygroscopic it is possible to dispense with the humidifying and drying sections of the apparatus.

16.6 METHODS FOR TESTING VESICANTS

The usual method for testing the vesicancy of an agent is to put a known amount of it on the skin of the forearm and to observe the results at a later time. The agent may be put on as either a liquid or a vapor; it may be either still or flowing.

16.6.1 Testing Vesicants as Liquids

The Edgewood Rods.^{6,13} One of the simplest methods for testing compounds for vesicant action involves the use of a series of stainless-steel rods of standard weight with tips varying from 0.6 to 2.68 mm in diameter.²² With the exception of the smallest rod, all of them deliver 0.022 to 0.029 mg of H per square millimeter. These rods, usually known as "Edgewood rods," are touched to the surface of a pad saturated with the vesicant and then applied to the skin. Liquids were used either undiluted or dissolved in diphenyl ether. Solids were also dissolved in diphenyl ether.

The method is simple and although all the material on the surface of the rod is not delivered to the skin, easily reproducible burns result from the use of a given rod with a given compound. In general the rods have not proved satisfactory for comparison of vesicants since a separate calibration is required for each compound tested. It is not possible to test ointments with these rods, since the droplet cannot satisfactorily be delivered to the surface of ointment-covered skin without breaking the covering. Further, the method is not desirable for compounds that react with steel, although it has been used with lewisite. Nonstandard sets of glass rods have also been made.

"Drod." It was necessary to devise some form of micropipet which would deliver small, known volumes of vesicant. Trevan³⁹ used a standard micrometer caliper to drive a 1-cc syringe. A modification²

called the Drod used a specially constructed micrometer head to drive the plunger of a $\frac{1}{4}$ -cc tuberculin syringe. A spring click bears on 12 longitudinal grooves on the barrel of the head, each click corresponding to 30 degrees of rotation and the delivery of about 0.2 cu mm of liquid. (The amount would be constant for each syringe, but commercial syringes are not interchangeable, being individually ground to fit. The diameters of the pistons, and hence the volume delivered, vary between syringes.) A 27-gauge needle, with the tip ground flat and square, is used to deliver the liquid.

The instrument is sturdy and portable. It delivers an accurately measured small dose, which is not so dependent on the physical properties of the agent as is the case with the Edgewood rods. This apparatus requires considerable time to fill, and the change from one vesicant to the other requires decontamination of the syringe and tip, making it unsuitable for use when many different liquids are to be handled in one day. The amount of liquid delivered per click is large, with the result that dilutions must frequently be used.

*The Modified Drod.*⁷ An attempt was made to modify the original Drod to make it more suitable. Several modifications were made on the driving head. It was redivided, so that a click occurred for each 7.5 degrees of revolution (48 clicks per revolution). The instrument then delivered 0.065 mg of H per click, instead of the original 0.2 mg. A 6-inch indicator disk, with 192 divisions, and a pointer arm attached to the head made it possible to split the clicks in half, and possibly into four. These are equivalent to 0.032 mg and 0.016 mg of mustard.

The $\frac{1}{4}$ -cc tuberculin syringe was retained. It is filled with mercury, which is used to expel the agent from a removable delivery tip. The syringe and screw are attached by a ground joint to a three-way stopcock. With the stopcock in one position, the agent in the tip may be expelled by turning the micrometer. With the stopcock in the other position, the tip may be filled or washed by liquid which enters from a side arm. A platinum or graphite surfaced stopcock is used to avoid fouling the agent.

It is possible with this modification to remove one vesicant, decontaminate the apparatus, and load another vesicant in less than 1 minute. It was found that dividing the clicks into four did not give reproducible lesions, but 0.032 mg of mustard, corresponding to half clicks, can be delivered quite accurately. This amount, although small, was not small enough

for some purposes. The increments were too coarse to discriminate between vesicants of nearly similar potencies.

Other Pipets. Capillary tubes have been used for the application of measured amounts of vesicant.²⁴ The capillaries were however rather fragile and the method is not adapted to testing large numbers of men. A device for blowing drops of measured size off a microburet tip was developed at Porton.^{31,32}

*The Benesh Micropipet.*¹³ In the Drod type of micrometer syringe the piston was of a diameter equal to the bore of the cylinder. To achieve a smaller displacement with the same pitch lead screw, it was necessary to reduce both bore and piston diameters. The $\frac{1}{4}$ -cc syringe already in use was the smallest available size. Micropipets capable of delivering smaller quantities of liquid have previously been described.^{37,38} Various features in their design were not, however, suited to vesicant testing.

The Benesh micropipet was based on a somewhat different displacement principle. The piston was a steel wire 0.0122 inch in diameter. This entered a mercury chamber through a Neoprene gasket. The volume of mercury displaced was equal to the volume of wire which entered the chamber, but since the piston worked by displacement it was unnecessary for it to be tightly fitted to a cylinder. This scheme avoided the difficulties of accurately machining such a small size hole. The wire piston is driven by a micrometer head, somewhat larger than usual, but of standard design. Twenty-five grooves are cut on the thimble actuating a spring click. The lead screw has the standard micrometer pitch of 40 threads to the inch. The dimensions of the wire and the pitch of the lead screw are such that each click ($\frac{1}{25}$ th revolution) advances 0.002 cu mm (2.5 gamma) of H.

The mercury chamber communicates with a removable tip, made out of capillary tubing. The end of the tip is optically polished. The instrument is mounted to move up and down on a rod screwed to a wooden base. The base forms the bottom of the carrying case, with the rod serving as a tie rod to hold the top and bottom of the case together. The instrument can be transported as easily as a compound microscope.

The apparatus is durable and simple. It has been found to give reproducible lesions. The principal defect is incomplete delivery of all the material advanced to the capillary tip. It is necessary that each subject's arm come in contact with the tip with the same pressure. The extent of loss due to the evapora-

tion of the compound between the time it gets to the tip and the time that it is applied to the subject is unknown. It is minimized by maintaining a regular, rapid rate of application. The instrument can best be used by a trained pair of operators, one operating the micropipet, the other holding the men's arms against the tip. A regular rhythm soon leads to both speed and accuracy.

It was found that the necessity for counting a number of clicks repeatedly led to personal error. An attachment was made for the pipet that made it possible to advance the desired amount in a single motion, rather than by counting a number of clicks. A brass plate with 25 holes equally spaced around the periphery was attached to the instrument. An index arm was attached by a ratchet to the lead screw. By placing taper pins in the appropriate holes any number up to 25 clicks can be delivered without counting.

Liquid Vesicant Cup.^{21j} Occasion arose to compare the action of HN3 as a liquid with saturated HN3 vapor. The vapor concentrations were set up in vapor cups (see Section 16.6.2). The apparatus employed for the application of liquid consisted of a small cup, 12 mm outside diameter and 8 mm inside diameter, with two capillaries leading from it. One capillary leading directly upwards from the cup, was attached to a safety flask and a charcoal column aspirator; the other tube, coming from near the base of the cup at a 45-degree angle, is connected with a three-way stopcock. A pear-shaped bulb with a small hole in the side is sealed to the vertical arm of this stopcock.

The liquid vesicant is placed within the bulb, and the stopcock is turned so that the vertical arm is connected with the cup. The cup is placed on the subject's arm, and the vesicant is drawn by suction out of the bulb, through the capillary, and into the cup until the area on the arm is covered with a continuous layer of vesicant. At the end of the exposure period 5 per cent hydrochloric acid is sucked through the instrument, followed by water; by applying the suction intermittently, the surface of the arm is flushed and decontaminated. In control tests with fat-soluble dyes all visible dye was removed within 5 seconds.

16.6.2 Testing Vesicants as Vapors

For proper evaluation of the vesicancy of a compound the vapor hazard must also be determined.

*Edgewood Vapor Cups.*¹⁰ One of the simplest ways of producing vapor burns is by the use of small glass

cups with a flat rim. A pad of filter paper or some other absorbent material is placed in the bottom and moistened with the liquid vesicant. The cups are then taped on to the arm of the subject for the desired length of time.

The amount of vapor (and its effectiveness) in the cups will vary as a result of the interplay of outside temperature, skin temperature, amount of moisture under the cup, and the presence or absence of sunlight on it. The actual concentration in the cup is undeterminable and may be changed by cooling or warming the cups. In addition to vapor burns, "rim burns" sometimes occur. These are the result of condensation of liquid agent on the lip of the cup. The use of these cups permits the application of approximately saturated concentrations of vapor.

Modified cups have been devised which permitted the application of subsaturation concentrations, provided for circulation of the vapor, and eliminated rim burns.^{17,18}

*The Vapor Train.*¹¹ Some of the objections raised to the use of the Edgewood cups are similar to those raised against the use of "static" chambers. A dynamic method of exposure was devised to overcome some of these difficulties.

This apparatus consists of the following essential parts. (1) A *bubbler* from which the *agent* is vaporized. (2) A *second bubbler* in which water is vaporized. (3) A *Y tube* that unites the streams of vapor-laden air from the two bubblers. (4) *Glass tubing* which is branched and rebranched to divide the vapor-air stream into four identical streams. This tubing, 20 mm inside diameter, is in several sections that are joined by 29/42 standard taper joints. (5) *Four applicator orifices*. Each may be described as an open cup with a delivery tube for conducting the vapor-laden air to it and a side arm that serves as an outlet. The cup is formed from a 24/40 male standard taper joint. The delivery tube, 8 mm inside diameter, enters the cup at the bottom through a ring seal and protrudes to within 3 mm of the upper, open end. The vapor-air stream, therefore, flows upward through the delivery tube, impinges upon the skin of the arm which a subject holds over the opening of the cup, and out through a side arm. The velocity of this jet is about 5 mph. (6) *Four stainless-steel adapters* for the applicators, each with an 8-mm hole in the center. Use of these adapters reduces the area of skin exposed and thus minimizes the severity of the resultant lesion. Each cap has a small ridge at its outer edge (1/32-inch deep) to prevent an arm from mov-

ing around during exposure. The caps are held in place by rubber bands. (7) A *branched and rebranched glass tube*, identical with (4) but used for uniting the effluent streams from the applicators. (8) A *tube* to conduct the combined effluent into a suitably ventilated duct. (9) A *sampling apparatus* to draw a measured volume of the effluent through a suitable absorber for determination of the analytical concentration of the vapor (see above). (10) Platforms upon which subjects rest their arms while holding them over the applicators. These are small tables of appropriate height with holes through which the applicators protrude about $\frac{1}{4}$ inch. The skin is thus held firmly against the cap of the applicator without any possibility of excessive pressure, and the arm rests comfortably during the exposure.

This apparatus, with a volume of 2 l and an air flow of 20 lpm, can be classified as a small, high-flow chamber. Concentrations of agent can be used up to saturation; the humidity of the air can be varied from dryness to saturation. Good analytical-nominal ratios are obtained. The apparatus is rapid and convenient to use.

Use of Dynamic Chambers for Body Exposures. Almost all of the standard type chambers in this laboratory have, at one time or another, been equipped for body exposures. The bodies of the animals are exposed to contaminated air, while their heads are in fresh air. A gasket around their necks prevents leakage of the noxious air and its inhalation. In one of the earliest methods¹ for use with a small smoke chamber the animals were placed in the chamber and provided with a manifold through which pure air circulated. It has been more common practice to

place the bodies of the animals in the chamber and let their heads protrude. The first chamber to have built-in provisions for body exposures was the 200-l chamber.⁶

Use of Wind Tunnel for Testing Vesicants on Man.^{21j} The wind tunnel (p. 285) is equipped with ports through which arms can be inserted perpendicular to the air stream. Since turbulent flow occurs, it was necessary to expose an annular space around the arm.

The arm was prepared for exposure by wrapping the hand and wrist to a point 5 cm above the distal end of the ulna with oilcloth sealed with adhesive tape. A piece of adhesive tape 2 inches wide was placed around the forearm leaving an exposed annulus of skin 1 cm wide between the wrist covering and the adhesive tape. Another piece of oilcloth covered the remainder of the forearm and elbow, leaving a second (proximal) annulus between the 2-inch tape and the elbow covering. To deliver two doses to the same arm the distal (wrist) annulus was left exposed for the whole exposure period; the proximal (elbow) annulus was kept covered with oilcloth except for the appropriate terminal fraction of the exposure period. At the end of the exposure the coverings were removed and discarded.

The use of the wind tunnel permits testing the relative efficiencies of aerosols and vapors at various wind speeds. Temperature and humidity of the air stream can be controlled only by controlling the temperature and humidity of the laboratory.

*The Great Lakes Man-Chamber.*²⁶ This apparatus for testing effects of vesicant vapor on masked men has been described in Section 16.2.2.

Chapter 17

PHYSIOLOGICAL MECHANISMS CONCERNED IN THE PRODUCTION OF CASUALTIES BY EXPOSURE TO HEAT

By *Alan R. Moritz*

17.1 INTRODUCTION

AT A MEETING called at the instigation of the Technical Division of the Chemical Warfare Service on March 22, 1944, certain deficiencies in the existing state of knowledge concerning the casualty-producing effectiveness of the flame thrower were discussed. Attention was called to the fact that, although both heat and the inhalation of irrespirable or poisonous gases probably contribute in varying degrees to these effects, little was known regarding their relative importance.

It was recommended that the physiological section of Division 9 of the National Defense Research Committee [NDRC] investigate the various mechanisms by which flame thrower action may cause disability and death. In this chapter are reviewed the studies that were made of the mechanisms by which excessive environmental heat may lead to early disability and death.

17.2 PILOT EXPERIMENTS TO EXPLORE CASUALTY-PRODUCING ATTRIBUTES OF GASOLINE CONFLAGRATIONS

A certain amount of general information concerning the thermal and chemical attributes of gasoline conflagrations was prerequisite to the planning of an experimental program. For the purpose of orientation, certain exploratory investigations were made of the rate, magnitude, and duration of the changes that occur in the temperature as well as of those that occur in the atmospheric concentrations of oxygen, carbon dioxide, and carbon monoxide incident to the burning of measured quantities of flame thrower fuel in both closed and ventilated spaces.

17.2.1 Experimental Procedure

A series of experiments¹ were accordingly undertaken in which gasoline was burned in a fireproof

room having a capacity of 14.4 cu m. The construction of the room was such that it could be either closed or ventilated at will. The fuel was poured into shallow metal pans which completely covered the floor, which measured 1.6x3 m. Approximately 4 liters were burned during each conflagration.

To measure the changes in temperature, 40 gauge iron-constantan spot-welded thermocouples were suspended in the center of the chamber. The thermoelectric potentials provided by the thermocouples were amplified by means of an electronic optical bridge circuit.¹⁸ It was found that the use of a split circuit is capable of amplifying a 1-mv input potentiometrically to a 5-ma output in less than 0.2 second. Since this amplifier was a null-point instrument, it was independent of all the electronic tube characteristics, of the intensity of the light beam focused on the photocell, and of the input resistance of the thermocouple leads. Two such amplifiers were constructed.

Two recorders were used. One was an Esterline-Angus recording milliamperemeter (5 mil, full scale) with a response time of 0.5 second. The other was a General Electric photoelectric recording milliamperemeter with a response time of 0.2 second. Both recorders had 12 inch per minute chart drives.

By means of a selector switch the sensitivities of the amplifiers were usually set so that a 40-mv input produced full-scale deflections of the recording pen.

Method of obtaining samples of atmosphere for gas analysis: Three long tubes, each having an internal diameter of 2 mm, extended from the outside to the center of the conflagration chamber. These tubes passed through the wall at the bottom, middle, and top of the room. Samples of 300 ml were withdrawn as desired by attaching evacuated flasks with ground joints to the ends of these tubes. The gas samples obtained in this manner were analyzed for O₂, CO₂, and CO by means of a standard Orsat apparatus.

17.2.2 Temperatures Developed during Gasoline Conflagrations

Unventilated conflagrations: In these experiments the door was kept closed during the fire. Oxygen depletion resulted in extinction of the conflagration in about 30 seconds after ignition. Approximately half of the gasoline contained in each pan remained unburned. When the door was opened following the premature extinction of the fire, the room was found to be filled with dense black smoke and there was a strong odor of gasoline.

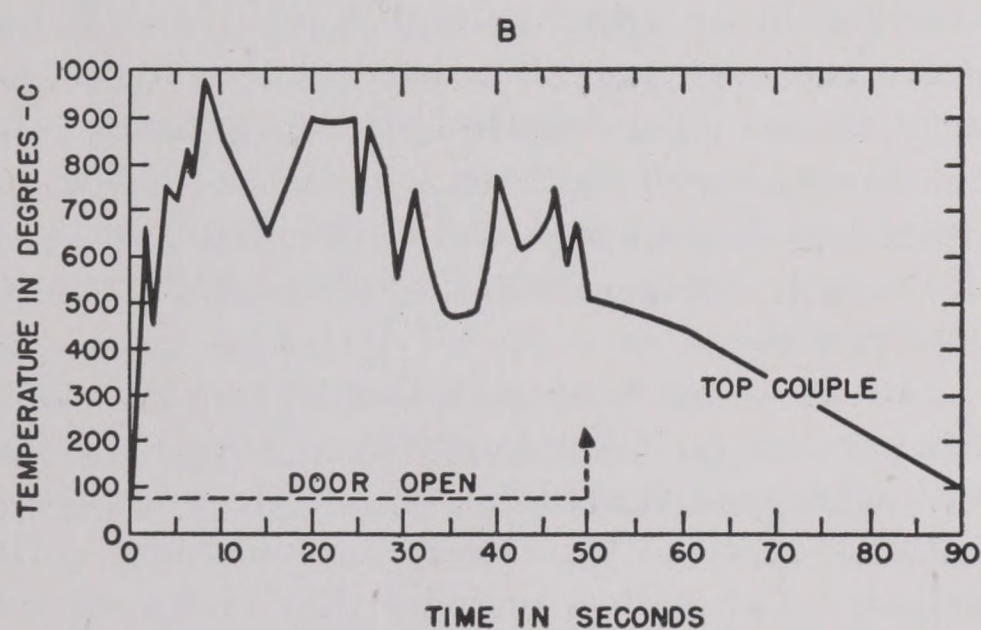
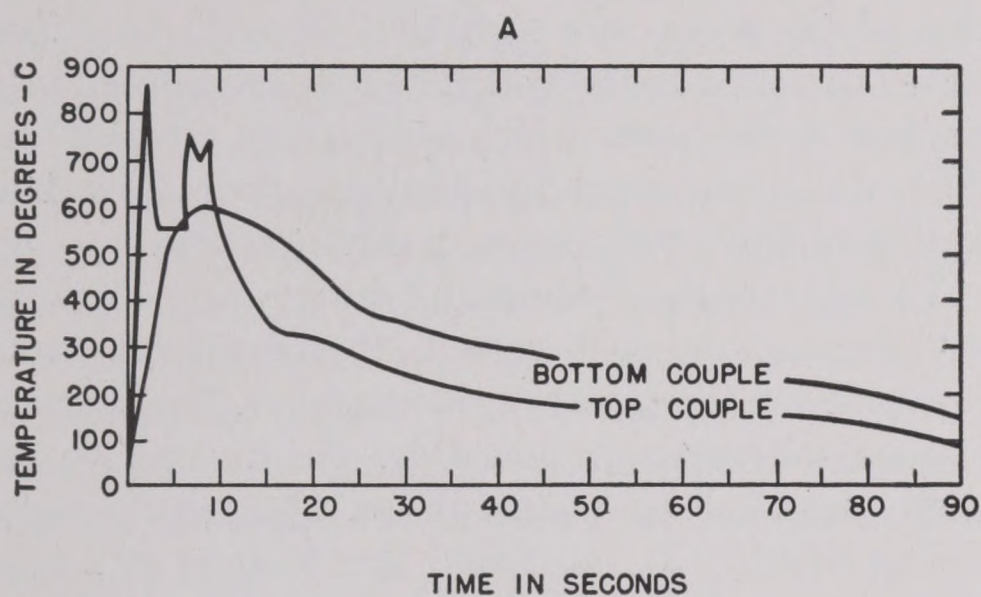


FIGURE 1. Continuous temperature recording during burning of gasoline in rectangular (6x10x10 ft) combustion chamber.

(A) No ventilation. Two thermocouples, one 5 ft and the other 3 ft above floor level. Distance from floor to ceiling was 10 ft.

(B) Room ventilated for 50 seconds. One thermocouple 5 ft above floor.

Figure 1A shows continuous temperature records provided by the two thermocouples, one of which was hung midway between the floor and ceiling in the center of the 3 m high conflagration chamber, and the other approximately 0.9 m above the floor.

Because of rapid convection currents, the upper thermocouple reached higher temperatures than did

the lower. The sharp peaks in the temperature curve of the upper thermocouple are also due to convection currents. The average temperatures recorded by the two thermocouples over a 30-second period were approximately the same, namely, about 500 C. At the termination of the combustion, the ambient temperatures fell rapidly and uniformly. The curves shown in Figure 1A are typical of all experiments in which the conflagration was unventilated.

Ventilated conflagrations: Figure 1B shows a continuous temperature recording of a thermocouple which was situated about 1.5 m above the floor during a conflagration in which ventilation sufficient to maintain complete combustion was provided.

The temperatures obtained were about the same as those recorded during unventilated conflagrations. The duration of the high-temperature plateau depended on the length of time that the door was left open. In the experiment in which the record shown in Figure 1B was made, the door was left open for 50 seconds.

17.2.3 Extrapolation of Experimental Temperature Changes to Conditions Likely to Prevail in Bunkers and Pillboxes Incident to Flame Thrower Attack

It was judged that the circumstances which prevailed in the experiments just described probably predisposed to the development of maximal temperature elevations. It is regarded as unlikely that higher temperatures would be developed in bunkers or pillboxes incident to flame thrower attacks in which gasoline was used as fuel. Due allowance should be made for the tolerance of commercial recording instruments in the interpretation of data pertaining to temperature changes in bunkers and pillboxes incident to field tests of the effectiveness of flame thrower equipment. Thermocouples of the usual size and potentiometers or millivolt meters of the usual period are not capable of following the rapid temperature fluctuations that occur in unventilated or incompletely ventilated gasoline conflagrations. Furthermore, temperature observations made by such apparatus may be lower than the actual temperatures obtained by as much as 500 C.

17.2.4 Exposures of Animals to Burning Gasoline

Adult dogs (6–8 kg) and young pigs (7–12 kg) were exposed in various ways to burning gasoline.

TABLE 1. Effects of temperature and combustion products resulting from gasoline conflagrations on animals.

Ref.	Conflagration		Site of thermal exposure			Inhalation of combustion products		Comp. (%) of air immediately after fire			Fate of animal		Blood after expt CO sat %
	Time sec	Avg Temp C	Body and face	Body only	Face only	With fire	After fire	O ₂	CO ₂	CO	Dead in 15 min	Survival for hours or days	
1	30	320	+	..	..	+	5 min	14.6	4.0	0.8	..	+	30
2	40	600	+	..	..	+	No	16.2	4.0	0.8	+	..	7
3	30	400	..	+	..	No	No	...	..	..	..	+	..
4	30	370	..	+	..	No	No	...	..	..	..	+	..
5	75	700	..	+	..	No	No	...	..	..	+	..	..
6	30	350	..	..	..	No	5 min	14.7	4.6	1.0	..	+	6
7	30	350	..	..	+	+	4 min	15.7	3.5	0.3	..	+	Trace
8	30	450	..	..	+	+	4 min	16.1	3.6	0.7	..	+	37
9	30	500	..	..	+	+	2 min	...	..	..	..	+	32

The animals were anesthetized by the intraperitoneal injection of sodium pentobarbital and fastened by asbestos tape to an iron frame situated in the center of the conflagration room 54 inches above the floor. The principal data pertaining to these experiments are included in Table 1.

COMBINED CUTANEOUS AND RESPIRATORY EXPOSURE

Animals 1 and 2 were exposed to the full effects (cutaneous and respiratory) of the burning gasoline. Throughout the entire exposure of animal 1, the door of the conflagration chamber remained closed. The fire burned out in about 30 seconds because of insufficient oxygen. The average temperature of the air surrounding the animal during this period was 320 C. The animal was allowed to breath the atmosphere of the unventilated room for 5 minutes after the fire was extinguished.

Samples of the atmosphere were taken for gas analyses as soon as the fire had burned out. The mean concentration of CO in the atmosphere was 0.8 per cent, and the oxygen concentration was 14.6 per cent. The CO saturation of a sample of the animal's blood taken 5 minutes later was 30 per cent. Although there was no indication that the fire had resulted in a dangerously low oxygen or a dangerously high CO₂ concentration, it did appear likely that the animal would have died of CO poisoning if it had remained much longer in the unventilated room.

Although animal 1 had been severely burned, it did not develop early shock, required several post-exposure injections of Nembutal to keep it quiet, and was beginning to become restless with returning consciousness when sacrificed 6 hours later. Its air passages contained an excessive amount of mucus but

there was neither clinical nor pathological evidence of significant thermal or chemical injury of the larynx, air passages, or lungs.

In the case of animal 2, the door remained open during the first 40 seconds of the conflagration, with the result that a larger amount of gasoline burned and a higher temperature was achieved and was maintained for a longer period of time than was the case in the first experiment. At the end of 40 seconds, the door was closed with the result that the fire was extinguished very soon thereafter. Samples of the atmosphere were then taken for gas analyses and the animal removed. This dog was moribund when removed to the open air. In view of the fact that the atmospheric concentration of CO was similar to that observed in the preceding experiment, it was surprising to find that the CO saturation of the blood was only 7.0 per cent. The explanation of this disparity probably lies in the fact that animal 1 breathed the atmosphere of the conflagration chamber for a total of 6-7 minutes, whereas animal 2 was moribund at the end of 2 minutes.

Two factors may have contributed to the extremely rapid death of dog 2. One is systemic hyperthermia caused by overheating of the blood as it circulated through the extensive superficial network of subcutaneous vessels. The other is respiratory obstruction due to pharyngeal edema. That a significant degree of hyperthermia had occurred was indicated by the finding of a rectal temperature of 41.2 C when the animal was autopsied 5 hours after the exposure. That obstruction to respiration may have contributed was indicated by the presence of severe burning of the mouth and pharynx with what appeared to be obstructive edema of the latter. The trachea and bronchi contained abundant mucus

mixed with carbon particles. The lungs were hyperemic.

The results of the first two exposures dealing with the effects on animals of burning gasoline indicated that even in circumstances considered to be particularly favorable to the production of CO and to the exhaustion of oxygen, the concentration of these gases was not sufficiently altered to cause unconsciousness or death within 5–6 minutes. Although the results of the two experiments were not construed as proof that neither fatal anoxia nor fatal CO poisoning could result from a gasoline fire, they did indicate that such an exposure can cause rapid death from thermal injury alone.

CUTANEOUS EXPOSURE

The next three experiments shown in Table 1 were undertaken to ascertain the effect of protecting the respiratory tract against heat and combustion products during the time that the body was being exposed. To investigate this question, animals 3, 4, and 5 wore tight-fitting asbestos-covered rubber masks through which a continuous stream of unheated air was circulated during their exposure to heat. The first two animals of this series (3 and 4) were exposed to an unventilated conflagration of about 30 seconds duration and average atmospheric temperatures of 400 and 370 C, respectively. Although both animals showed extensive burning of the skin, they survived the immediate effects of heat and were in reasonably good condition when killed 6 hours later. In the case of animal 5, the door of the room was left open for the first minute of the fire and for 65 seconds the temperature of the room was in excess of 400 C. Within 15 seconds after the door was closed the fire went out and the animal was removed. This animal died immediately on reaching the open air and showed severe burning of all the body surface except where the skin had been protected by the mask.

These experiments provided evidence that a relatively brief (75 seconds) exposure of the skin to a sufficiently high temperature could cause almost immediate death independently of other factors.

RESPIRATORY EXPOSURE

The last four experiments shown in Table 1 were undertaken in an attempt to investigate further the effects on animals produced by the breathing of the combustion products of a gasoline conflagration. In each experiment the door was kept closed throughout

the entire conflagration. By this procedure postconflagration mixing of outside air with the combustion products was reduced to a minimum. The skin of the body was protected against excessive overheating by enclosing the animals to the neck in a heavy asbestos sack. With the exception of No. 6 the animals were free to breathe the burning gases and hot air during the fire as well as the smoke which remained in the chamber after the fire. Dog No. 6 breathed outside air circulated through the mask during the fire; as soon as the temperature in the room had dropped to 200 C the mask was detached by remote control and for the next 5 minutes only the hot smoke and air of the combustion chamber were available for respiration.

None of these four animals showed either clinical or pathological evidence of thermal injury of the air passages or lungs. Two of them (6 and 7) may have held their breath throughout most or all the exposure period. That animals No. 7 and 8 breathed during some of the time that they were in the combustion chamber is indicated by their carboxyhemoglobin concentrations of 37 and 32 per cent, respectively. It is possible, of course, that even these two animals held their breath during the conflagration and acquired their carbon monoxide by breathing during the interval between the time that the fire went out and the time that they were removed from the chamber.

17.2.5

Summary

The ignition within a simulated pillbox or bunker of a well-spread layer of gasoline leads within 10 seconds to a temperature rise of between 800 and 1000 C. The duration of such a conflagration and the temperature increase caused by it varied according to the oxygen supply and the fuel. In a closed room having a capacity of 14.4 cu m and measuring 1.6x3x3 m, the fire was extinguished within 20 seconds and considerably less than 250 ml of gasoline was consumed for each cubic meter of air space. For approximately 10 seconds of the burning time the temperature fluctuated between 500 and 900 C. If such a room is ventilated, the initial temperature rise is similar to that which occurs in a closed room, but the fire continues to burn until the fuel is exhausted, resulting in a temperature fluctuation of between 500 and 1000 C. In both instances, convection currents established by the conflagration resulted in marked fluctuations in the temperature at any given place within the room. During the period of rapid com-

bustion the temperatures were highest near the ceiling and lowest near the floor.

In none of the experiments conducted in this particular type of conflagration chamber did the oxygen content drop below 14 per cent. The carbon dioxide level did not rise higher than 5 per cent nor the carbon monoxide level above 1 per cent.

The most important information gained from these exploratory experiments was the observation that animals as large as dogs and pigs when exposed to this kind of a conflagration for more than 30 seconds may receive injuries that are almost immediately fatal. Such fatalities were not necessarily contributed to by asphyxia, carbon monoxide poisoning, or inhalation of flame. It appeared that the rapid death may result from systemic disturbances caused by the impact of heat energy on the surface of the body. It was obviously in order to conduct additional and better controlled experiments to investigate the physiological mechanisms concerned in the production of casualties through the thermal effects of flame thrower attack.

17.3 BASIC CHARACTERISTICS OF HEAT AND HEAT TRANSFER^a

It could be inferred from the results of the pilot experiments reported in the preceding section that heat independently of other factors was an important, if not the most important, casualty-producing attribute of flame thrower action. This being the case, consideration should be given to the nature of heat and to the factors which determine the transfer of heat from one medium to another and from one place to another within the same medium.

17.3.1 Theoretical Considerations

THE NATURE OF HEAT

The concept of temperature rises from the sensations of hotness and coldness. Experience has shown that when two or more substances of different temperature are kept free of all outside disturbances, the hotter bodies will get colder and the colder bodies hotter; and that ultimately these substances will reach a state of complete thermal equilibrium (identical temperature). The hotter bodies are said to have lost heat, and the colder bodies are said to have gained heat. This concept of heat becomes quantitative by defining a unit of heat, the calorie, as the

amount of heat gained by a 1 g of liquid water under atmospheric pressure when the temperature increases from 14.5 C to 15.5 C.

This gain in heat, which is discernible through a rise in temperature, is associated with an increase in the intra- and intermolecular motion. Thus heat can be considered as the energy stored in a substance by virtue of the state of its molecular motion. Certain manifestations of this increase in energy are readily observable, for example, melting, vaporization, decomposition, alteration in rate of diffusion and in chemical reaction.

Beside the definition of a calorie, there are other physical concepts pertaining to heat which are requisite to an understanding of the general problem of thermal injury.

HEAT CAPACITY

Heat capacity or specific heat of a substance is the amount of heat which is required to raise the temperature of the substance 1 C.

The importance of heat capacity (C_p) in thermal injury is readily seen by considering the respective injury propensity of 1 g of water ($C_p = 1.00$) and 1 g of silver ($C_p = 0.06$) both at 100 C placed in contact with 1 g of thermally insulated skin ($C_p \approx 0.7$) at 35 C. After equilibrium is reached in the former case, the temperature of the skin is increased to 73 C, whereas in the latter case it is increased only to 42 C.

It is apparent that, if the skin were to equilibrate rapidly enough when placed in contact with a hot body, there is insufficient heat in 1 g of silver at 100 C to produce injury to 1 g of skin. Actually, of course, the skin, because of its thermal insulating properties, does not equilibrate rapidly enough and the portion of skin nearest the silver does reach a sufficiently high temperature to produce injury before thermal equilibrium is reached. Hence another physical property of importance is heat transfer.

HEAT TRANSFER

In the experiments to be described heat was transported to the skin by three mechanisms: namely, convection, radiation, and conduction. In the case of convection and radiation, heat reaches the skin under such circumstances that the heat uptake is primarily determined by the heat source. In the case of conduction, the amount of heat absorbed by the skin is primarily determined by the properties of the heat absorber, namely, the skin itself.

^a By F. C. Henriques, Jr.

CONVECTION

Convection is the mechanism by which hot air transports heat to a cooler surface because of the eddying currents that arise. The air velocities of the eddy currents are about 1.6 km per hour. An equation has been developed for the transfer of ambient heat by natural convection from a large envelope of hot air surrounding cylindrical objects about 30 cm in diameter.^{29,35} This equation shows that q , the caloric uptake per minute per square centimeter of surface, can be expressed as follows:

$$q = 0.0026(T_a - T_s)^{\frac{5}{4}} \quad (1)$$

where T_a is the air temperature in C and T_s is the surface temperature in C.

Thus, with a skin temperature of 40 C, air at 100 C and 400 C will transport to the skin about 0.4 and 4 cal/cm²/min. It is also apparent that as this heat is absorbed by the skin the surface temperature of the skin will rise and the caloric uptake of the animal will decrease with time.

It is of interest to compare with this the caloric uptake rate of skin at 40 C when an atmosphere of steam maintained at 100 C is substituted for the air. Under these conditions, about 300 cal/cm²/min would be absorbed by the skin³⁵ if the surface temperature could be maintained at 40 C. This 800-fold increase in caloric bombardment as compared with that produced by air is due to the latent heat of condensation of steam. This, of course, is why steam is an enormously greater hazard than hot air in the production of heat injury.

RADIATION

All substances give off heat in the form of radiant energy in amounts that are predetermined by the surface temperature of the substance. When this radiation impinges upon another body, a certain fraction is absorbed and changed into heat. Thus, if two substances at different temperatures are placed in an enclosure, there is a continual exchange of energy, the hotter body radiating more energy than it absorbs and the colder body absorbing more heat than it radiates.

In the special case of an animal completely enclosed in a large box of source temperature T_r , the caloric uptake rate q of the animal, due to this interchange of radiant energy between the skin and the wall of the box, is expressed by the following equation.^{30,35}

$$q = sef[(T_r + 273)^4 - (T_s + 273)^4] \quad (2)$$

where s is the radiation constant and is equal to 8.2×10^{-11} calorie, per square centimeter per T^4 per minute, e is the effective emissivity of the hot walls of the box, and f is the absorptivity of the skin to radiation emitted at T_r . Under experimental conditions to be described, the product ef can be taken as about 0.8. Thus, when the skin temperature is 40 C, the hot walls at 100 C or 400 C will radiate to the skin about 0.7 or 13 cal/cm²/min, respectively.

CONDUCTION

Conduction is defined as the transfer of heat from the hotter portion of a substance to a colder portion of the same substance, or from a hot body in physical contact with a cold body, where in each case there is no appreciable displacement of any of the molecules comprising these substances. It is the latter restriction that differentiates conduction from convection.

In certain experiments to be described heat was conducted from either a hot solid or a hot liquid to the skin. In these experiments, the purpose of both the solid and liquid heat source was to maintain the temperature of the skin surface at a predetermined constant value and hence the conduction of heat through the heat source need not be considered. In the hot air experiments, thermal conduction through air is small as compared with convection, and this small contribution is included in equation (1). Thus it is only necessary to consider conduction of heat through the skin.

In all cases of heat flow by conduction, a temperature gradient must exist within the substance. If this temperature gradient varies with time, the rate of heat flow will also vary with time. The type of heat flow where temperature is a function of both position within the body and time is called heat conduction in the unsteady state. Heat conduction in the steady state refers to all cases where the temperature at any point within a substance does not depend upon time. Under these conditions the amount of heat flow through the medium is determined by this temperature gradient and the ability of the body to conduct heat (thermal conductivity). The latter case will be considered first. The equation for steady-state heat conduction inside a rectangular homogeneous body is based upon Fourier's law^{1,11} and is as follows:

$$q = \frac{K}{L} (T_s - T_o) \quad (3)$$

where K , the thermal conductivity, is expressed in

calories per minute, per square centimeter perpendicular to the direction of heat flow per unit temperature gradient in C per centimeter length of path. L is the path length through which the heat flows and T_s and T_o are the temperatures in C at the beginning and end of the path, respectively; q has been previously described.

This equation permits the experimental determination of the *in vitro* thermal conductivity of the four respective sections of tissue, namely epidermis, dermis, fat, and muscle, and also of any combination thereof.

GENERAL THEORY OF HEAT FLOW THROUGH SKIN

By making use of the preceding brief definitions of the various physical factors involved in the transport of heat to and through the skin it is possible to consider how the application of heat affects the time-temperature relationship within a given skin site. It is apparent that in order to make heat flow inward from the skin surface it is necessary to raise the temperature of the skin surface to an extent that overcomes the normal existing gradients. This can be accomplished by means of an external source of heat through conduction, convection, or radiation. Once the skin surface temperature is sufficiently high, the heat will start to flow inward, resulting in a general rise in temperature within the skin site.

This initial heat flow inward (and thus the rate of temperature rise within) will depend primarily upon two physical factors: namely (1) the heat capacity of the skin or the ability of the skin to absorb the heat, and (2) the thermal conductivity of the skin or the ability of the skin to transport the heat. After a certain interval of time the amount of heat entering the skin site will be balanced by the amount of heat leaving the skin site, and the skin will be "heat-saturated." In this state the new temperature distribution within the skin site will become invariant with time and the amount of heat flowing through the skin will depend only upon (2) and the skin surface temperature.

It is to be recognized that this picture involves not only the solution of the steady state of heat conduction but also the solution of the initial unsteady state of heat flow. In order to solve even the "idealized" picture, it would be necessary to know the initial temperature gradients within the tissue, the thicknesses, densities, thermal conductivities, and heat capacities of the various layers, and the skin surface temperature as a function of time.

The solution of such a problem involves the following Fourier heat equation:¹¹

$$\frac{K}{\rho C_p L^2} \left(\frac{d^2 T_{xt}}{dx^2} \right) = \frac{dT_{xt}}{dt} \quad (4)$$

where T_{xt} is the temperature time, t , at a distance, x , within the skin measured from the skin surface. ρ is density, and the remaining symbols have been previously defined.

The solution of equation (4) subject to these conditions is exceedingly complicated. Yet superimposed upon this are the numerous indeterminate *in vivo* factors which arise when we go from the idealized picture to the living animal. It is useful to enumerate the most important of these various indeterminate factors.

1. Site variations in the respective thickness of epidermis, corium, fat, and muscle.
2. Variation of existing temperature gradients within the skin with respect to time and/or position of site.
3. Unknown average rate of blood flow through the various skin layers, and the unknown variations of the unknown rate of flow with respect to position of site and temperatures within the site.
4. The appearance of edema fluid in variable quantities which brings forth indeterminate alteration in the density, heat capacity, thickness, and thermal conductivity of the various layers of skin so affected.

It is obvious from this discussion that any general solution of the time-temperature relationship within a skin site, when heat is applied, is not possible. However, with certain of the experiments to be described later in detail, it is possible to derive to a first approximation the time-temperature relationship in the layer of basal epidermal cells. These experiments were either (a) so conducted to bring immediately to, and maintain the skin surface at, a predetermined temperature level until the threshold of irreversible epidermal injury was reached, or (b) the entire animal was completely surrounded by an envelope of ambient and radiant heat. These experimental conditions at the boundary of the skin surface and source of heat are expressed by the following equation:

$$q = H(T - T_s) \quad (5)$$

where q and T_s have been previously defined (see Section 17.3.1 under "Convection"). T is the temperature of the heat source in C and H , in cal/cm²/min, is known as the heat transfer coefficient. Conditions

under experiments (a) were tantamount to an infinite heat transfer coefficient ($H = \infty$); with experiments (b), the heat transfer coefficient is finite and the numerical value readily obtained by combining the radiant and ambient contributions to heat transfer coefficient as computed by equations (1) and (2), respectively. In order to solve equation (4) under the boundary condition expressed by equation (5), it is necessary to assume that the ratio of the total tissue thickness to the epidermal thickness (approximately 80μ) is infinite rather than finite. This assumption will lead to slightly longer time intervals for "heat saturation" of the epidermis than are to be experimentally expected. The integration⁶ of equation (4) under the above conditions results in equation (6).

$$\frac{T_s - T_t}{T_s - T_o} = \theta \left[\frac{\gamma}{\sqrt{t}} \right] e^{\frac{HL}{K} [1 + (HLt/4\gamma^2 K)]} \left\{ 1 - \theta \left[\frac{\gamma}{\sqrt{t}} \left(1 + \frac{HL}{2K\gamma^2} \right) \right] \right\} \quad (6)$$

where

$$\theta(Y) = \frac{2}{\sqrt{\pi}} \int_0^Y e^{-x^2} dx \quad (6a)$$

and γ is computed by means of equation (6b).

$$\gamma = \frac{L}{2\sqrt{\frac{K}{\rho C_p}}} \quad (6b)$$

T_t is the temperature of the basal epidermal cells at the time t in seconds. T_s is the temperature of the heat source. T_o is the temperature of the skin surface previous to the exposure to heat. L is the distance of the basal cells from the skin surface. ρ is the density of the basal epidermal layer. The other symbols have been previously defined and are experimentally determinable. The integral that defines $\theta(Y)$ (equation 6a) is respectively equal to $\sqrt{\pi}/2$ and zero when Y is infinite ($t = 0$) and Y is zero ($t = \infty$). For other values of Y , the numerical value of the integral is tabulated.³⁹

The time-temperature relationships at the basal epidermal layer during an exposure of the animal to a source of constant ambient and radiant heat are evaluated by means of these equations in Section 17.3.2 (see also Section 17.9.2 under "Measurement of Heat Transfer").

In the experiments in which the skin surface was brought immediately to and maintained at a predetermined constant temperature, H , the heat trans-

fer coefficient, is nearly infinite, and equation (6) reduces to

$$\frac{T_s - T_t}{T_s - T_o} = \theta \left(\frac{\gamma}{\sqrt{t}} \right) \quad (6c)$$

where, as before, θ is given by equation (6a). It is to be noted that in this case T_s can be taken as the skin surface temperature during the entire heat exposure, since the temperature of the heat source is identical with the surface temperature once heat exposure begins.

It is to be noted that equation (6c) results in a basal layer temperature which becomes, after a certain time interval, essentially identical with the skin surface temperature. Actually, a small but finite temperature gradient will always exist between the surface and the basal cell layer. This steady-state gradient can be experimentally determined by means of equation (3), and the true temperature of the basal layer can be quite accurately computed for any time t by using equation (6c) until the steady-state temperature obtained through equation (3) is reached. Computations using equation (6c) to ascertain basal epidermal temperature are given in Section 17.3.2, and the experimental justification for this theory will be considered in Section 17.6.5 (see also 17.6.6).

17.3.2 An Experimental Investigation of Quantities Involved in Both Steady and Unsteady State of Heat Conduction through Skin^{3a}

It is apparent that certain types of special apparatus were necessary for the evaluation and assessment of the various physical factors involved in the time-temperature relationship to thermal injury. The description of these apparatuses will now follow in detail.

HEAT CAPACITY APPARATUS

The apparatus used for the determination of the heat capacity of the various skin layers need not be described in detail since these specific heats were determined by the well-known method of mixtures.⁴⁶ Briefly, this procedure consists of heating a known weight (about 10 g) of tissue in a brass container to 100 C and rapidly dropping it into a water calorimeter. The heat capacity of the tissue was readily computed from the temperature rise of the water as measured with a Beckmann thermometer.

AUTOMATIC ENERGY RECORDING APPLICATOR

In order to measure the rate at which heat energy was taken up by the skin during the entire exposure period at any predetermined skin surface temperature, the following apparatus was constructed to simulate an infinite source of heat at any given temperature.

The effect of bringing the skin in contact with a source of heat having infinite capacity and constant temperature is shown schematically in Figure 2. The temperature of the surface of the skin immediately reaches and is maintained at the temperature of the heat source. The rate of caloric uptake by the skin at the time of the initial contact is essentially infinite and as the skin approaches its new state of temperature equilibrium the rate of energy transfer diminishes and finally reaches a nearly constant value. Thus the curve representing rate of energy transfer is similar to that shown in Figure 2.

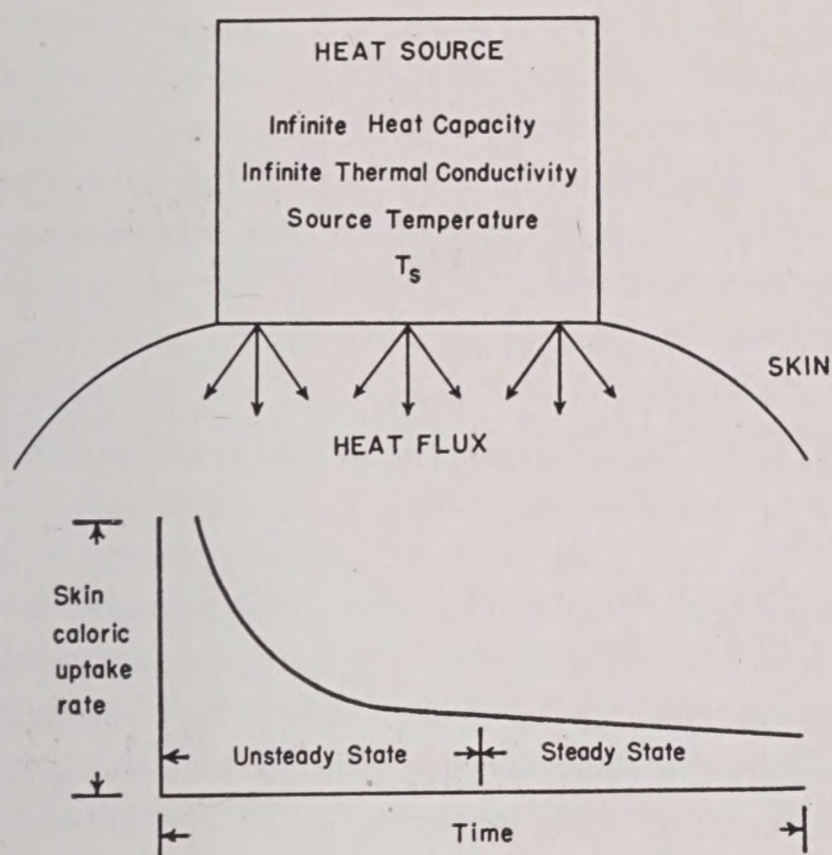


FIGURE 2. Diagrammatic representation of rate of caloric uptake of skin from heat source at constant temperature, of infinite thermal conductivity and of infinite heat capacity.

The steady state of caloric uptake is a measure of the thermal conductance of the skin. The unsteady state is a measure of the ratio of the thermal capacity to the thermal conductivity of the skin.

Unless the animal were completely enclosed by an infinite source of heat, there would be considerable lateral spread of energy from the application area (see Figure 2). It was apparent, however, that because of lateral spread the skin in the center of the application area would, under certain conditions,

gain as much heat from the surrounding area as it would lose to it. Thus the caloric uptake from the central area of the source (Figure 2) would be a measure of the perpendicular flow of energy through the directly subjacent skin if a sufficiently large surrounding area were in contact with the same or a similar source of energy.

A scale drawing of the caloric applicator is shown in Figure 3A. It consisted primarily of three separate parts — a crown, a brim, and an applicator disk. The crown and brim were brass, whereas the applicator disk was copper. The three units were maintained at the same constant temperature by independent electrical heating units. The temperature of the crown and brim were controlled manually by means of General Radio Variac transformers. The purpose of the crown was to prevent any leakage of heat from the applicator disk except via the exposed face. The brim compensated for the lateral spread of the heat from the surface of skin directly underneath the applicator. The applicator was heated by means of an auxiliary electronic apparatus which automatically recorded the wattage required for continuous maintenance of the face of the applicator at a specified temperature T . The temperatures of the crown, brim, and disk were measured by means of three calibrated 10-mil iron-constantan Fiberglas duplex (Leeds & Northrup) thermocouple wires. The wire heating units were of single, silk-insulated No. 40 manganin wire (negligible temperature coefficient of resistance). This wire was held in the indicated spiral grooves with a thin coat of glyptal. Copper lead wires were soldered to the ends of the manganin and the joints were electrically insulated from the metal parts with fine glass bushings. The electrical resistances of the brim, crown, and applicator were 390, 277, and 71.75 ohms respectively.

Three fine phonograph needles rigidly located the applicator disk inside the crown. The disk was held firmly against the needles by a rubber bushing under compression. A steel spring, the tension of which could be regulated by a hard rubber screw, controlled the pressure of the applicator against the skin. This pressure could be set between 5 and 50 g/cm². Guides served to keep the numerous lead and thermocouple wires apart, so that the pressure regulation was reproducible. The two lead wires to the heating unit of the applicator were held fast against the sides of the Fiberglas thermocouple wire by wrapping with thread for the first 10 cm and then with scotch tape.

gave a continuously variable control of the power output of the plate circuit. The 50-megohm resistance shunting the photocell added stabilization to the circuit. The 250- $\mu\mu\text{f}$ variable condenser "tuned" the phase angle of the grid circuit to the best operating conditions. These conditions were that a 4-mm deflection of the light beam reflected from the galvanometer would give full control of the plate wattage.

The purpose of the capacities and chokes in the plate circuit was to filter the pulsating thyatron output into steady direct current. The values of the condensers and chokes were necessarily large because of the high current requirements of the applicator heater. Oscillograph tests showed no appreciable ripple current in the filtered output. The wattage or caloric input rate into the applicator heater was measured with an appropriately shunted Esterline-Angus recording milliammeter (5 mil, full scale and $\frac{3}{4}$ to 12 inch per minute chart drive). Six scales were provided by a selector switch with full-scale deflections of 1, 2, 5, 10, 20, and 50 cal/min/cm² of applicator surface area respectively. The highest value corresponded to a filtered output of 36-v across the applicator heater terminals.

Operation. In use the galvanometer zero was set to provide sufficient illumination on the photocell to generate about 1 cal/min inside the applicator.

The potentiometer was now set to the predetermined millivoltage (temperature). By turning off and on the low-sensitivity shunt button of the type K potentiometer the photocell was kept fully illuminated until the galvanometer started to deflect in the opposite direction. The high-sensitivity button was now locked down and the instrument was on automatic control. Thus, if the temperature of the applicator face as measured by the applicator thermocouple tended to get either hotter or colder, the galvanometer mirror moved in a direction that either decreased or increased the illumination on the photocell, which in turn either decreased or increased the wattage through the applicator. Thus by means of the thermocouple in the face of the applicator the output of the thyatron tube was thermally "locked" to a predetermined temperature of the applicator face.

The sensitivity of the galvanometer was set to give a deflection of 4 mm for an 0.1 C change in temperature. This deflection was sufficient to produce the maximum available power of 50 cal/cm²/min. This was the maximum sensitivity that could be obtained

without producing periodic heating and cooling of the applicator face (slow oscillations of the recorder tracings of caloric uptake rate). These oscillations in the power output were due to the short but finite time for the heat generated in the heater wire to affect the thermocouple.

The heat losses of the applicator disk under the conditions of usage were determined by placing the disk and brim on a "perfect" insulator. The perfect insulator consisted of a flat-bottomed, thin glass cone which was silvered on the inside, pumped out to 10^{-7} mm of mercury while being heated to 450 C for 8 hours, and then sealed off. All heat losses from the inside surface of the glass were prevented by the bright silver surface (no radiant loss) and the vacuum (no molecular heat conduction). Lateral heat loss through the glass was prevented by maintaining the brim at the same temperature as the applicator disk.

Heat losses from the applicator disk were determined at two temperatures, namely 45 and 60 C. The results are given in Table 2.

TABLE 2

Exp	Temperature C			Applicator disk heat loss in cal/cm ² /min
	Crown	Brim	Disk	
a	45	45	45	0.020
b	45	Not heated	45	0.45
c	60	60	60	0.035
d	61	60	60	0.000
e	59	60	60	0.080
f	60	Not heated	60	1.1

These data showed that when all three units were heated to the same temperature the heat loss of the disk was trivial as compared with the caloric uptake of the skin at similar temperatures (see Table 5). The slow rate of heat transfer from the crown to the applicator disk was indicated by comparison of experiments *c*, *d*, and *e*. These data showed that the exact setting of the crown temperature was not critical. A comparison of the data *a*, *b*, *c*, and *f* showed the importance of the brim in preventing lateral heat leakage from the applicator disk.

NEEDLE THERMOCOUPLE FOR DETERMINING TISSUE TEMPERATURE BENEATH SITES OF CUTANEOUS EXPOSURE

It was desirable to be able to measure the temperature of the tissue at various distances beneath the surface of the skin before, during, and after exposure to heat. For this purpose a needle thermocouple was

constructed by threading a single silk-insulated 3-mil constantan wire through 4 feet of a No. 27 gauge trochar. The bimetallic junction was then made by honing down the end of the trochar and wire to a 45-degree angle; this removed the silk insulation from the constantan wire and permitted it to be surface soldered to the steel hypodermic needle. Through experimentation it was found possible to insert laterally a No. 22 gauge trochar along the natural cleavage plane of the dermis-fat interface, until a point directly underneath the surface area to be exposed was reached. Then the No. 27 gauge thermocouple needle was inserted into the No. 22 gauge trochar until skin resistance could be perceived, and the No. 27 gauge couple was withdrawn about 1 cm. After the heat exposure was terminated, the skin was cut to the needle depth and the distance from the muscle-corium interface to the skin surface was ascertained with a depth gauge. This depth before the application of heat was ascertained by a control experiment on a neighboring site.

The thermal emf of the steel-constantan couple was read on a Leeds & Northrup Type K2 potentiometer and high-sensitivity galvanometer. The steel-constantan emf seemed to be very reproducible in this temperature range 0 to 80 C. It was about 30 per cent lower than the iron-constantan emf. Temperature differences of 0.1 C were readily determined.

THERMOCOUPLE FOR MEASURING SURFACE TEMPERATURE OF SKIN

The surface temperature of skin exposed to air depends upon two factors, namely, the rate at which heat reaches the skin surface from the underlying tissue and the rate at which the skin surface loses heat to the atmosphere. When the surface temperature of the skin reaches a steady state, these two rates must be identical.

The use of the usual insulated thermocouples¹⁹ for the measurement of skin temperature necessarily alters these conditions. Upon first applying an insulated thermocouple, no matter how perfect the insulation, the temperature measured will be considerably lower than the true surface temperature because of the relatively high heat capacity of the insulator. When a steady state of temperature is finally reached (in some cases a matter of hours), the temperature recorded by the insulated couple must be greater than the true skin temperature, since the skin site is no longer losing heat directly to the air. Thus an accurate measurement of surface tempera-

ture by any apparatus similar to that just described would be fortuitous.

A thermocouple for measuring the surface temperature of the skin in this investigation consisted of a bare 2 mil iron-constantan junction. The 2-mil wires were prepared by dissolving (by nitric acid) the ends of 15-mil iron and constantan thermocouple wires (Leeds & Northrup) for a distance of 5 mm. The reduced ends of the two wires were then soldered end to end and stretched tightly by means of a bow made of brass tubing (see Figure 4). The heat capacity of the junction was trivial as compared with that of the skin.

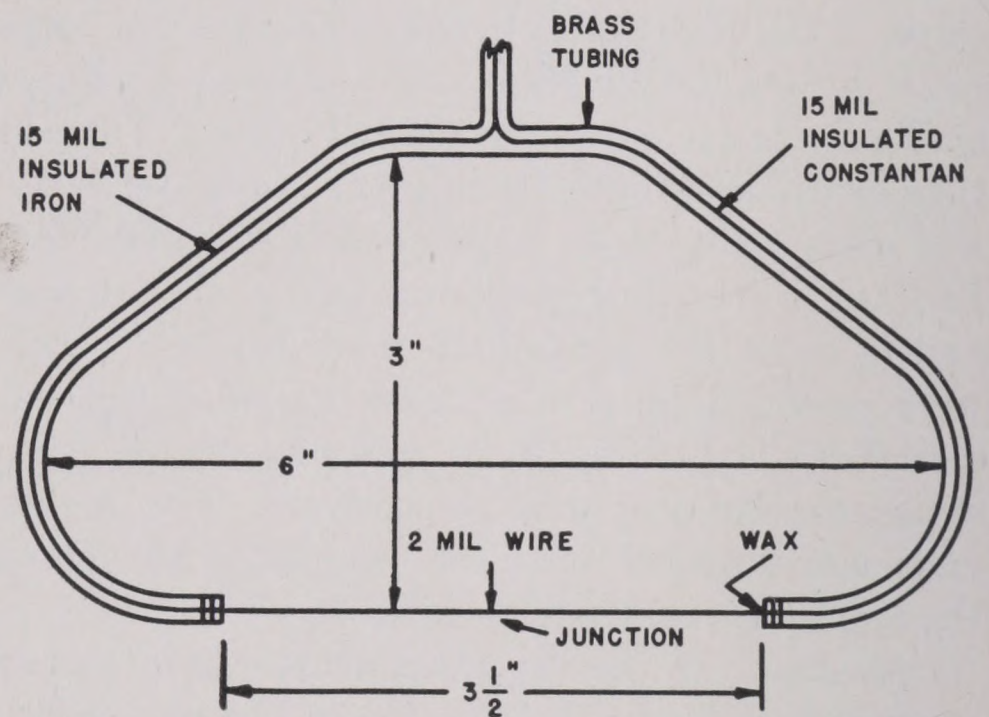


FIGURE 4. Thermocouple for measuring skin surface temperature.

In use the junction was placed on the skin for lateral contact and after 10 seconds a reading was made. The couple was then completely surrounded by skin by pinching the neighboring epidermis and a second reading was taken within 5 seconds. Numerous such pairs of readings were recorded and in no case has there been any significant difference between the temperature of lateral and that of circumferential contact. Thus a bare fine wire rapidly reaches skin temperature (10 seconds) when it is in contact with the skin.^b

The sensitivity of an iron-constantan thermocouple is such that with a Leeds & Northrup Type K2 potentiometer and high-sensitivity galvanometer

^b A theoretical objection to the unprotected or bare wire junction has been that it is partially exposed to the air and thus will reach a temperature somewhere intermediate between the air and the skin temperature. It should be kept in mind that the skin is also exposed to air. At normal air temperatures, the heat transfer coefficient for both wire and skin to air is quite small. Since the heat capacity of a fine wire is small and its thermal conductivity high, one would expect the wire rapidly to attain true skin temperature.

temperature differences of 0.05 C were readily measured.

DETERMINATION OF HEAT CAPACITY OF FOUR PERTINENT TISSUES (*in vitro*)

The heat capacity of pig epidermis, dermis, fat, and muscle were determined on approximately 10-g samples of each tissue by the procedure given in Section 17.3.2 under "Heat Capacity Apparatus." Determinations were made on each tissue of two 10-kg pigs. In order to obtain pure epidermis for these determinations the following method was used. After the hair was shaved as closely as possible, the pig was immersed in water at 55 C for about 1 minute, then removed, and the skin was carefully dried. It was then possible to remove strips of pure epidermis by scraping with a knife. The remaining tissues were readily obtained in a relatively pure state by dissection.

The values of the heat capacities of these tissues are given in Table 3.

TABLE 3. Heat capacity of pig tissue in calories per gram per C.

	Epidermis	Dermis	Fat	Muscle
Heat capacity	0.887	0.785	0.538	0.890
	0.845	0.753	0.573	0.926
Average value	0.86	0.77	0.55	0.91

In view of the similar heat capacities of dry tissue, the above variations of the different tissues are probably due to water content of tissue. In this respect the high value for pig epidermis (0.86) is understandable since it was found experimentally that the water content, in spite of the presence of the cornified layer, averaged about 76 per cent.

DETERMINATION OF THERMAL CONDUCTIVITIES OF TISSUES (*in vitro*)

The experimental determinations of thermal conductivities of pig epidermis, corium, fat, and muscle, were based on equation (3) of Section 17.3.1. The respective tissues were placed on a copper cylinder 2 inches in diameter and 4 inches high. The automatic energy recording applicator was now placed over and in contact with the tissue. Thus when the tissue became "heat-saturated," the knowledge of the caloric input into the tissue, the temperatures of the tissue-applicator (approximately 48 C) and tissue-cylinder (approximately 30 C) interfaces, and the thickness of the tissue permitted the computation of

the thermal conductivity. The temperature of the tissue-cylinder interface was measured by means of an iron-constantan thermocouple soldered into the face of the copper cylinder. The average tissue thickness was determined by measuring the distance of the face of the applicator from the face of the cylinder. The thermal conductivities of all the tissues except epidermis were obtained by this procedure, since in view of the epidermal thinness the above method was not adaptable.

The method of difference was used with epidermis. A section of well-shaved skin tissue consisting of dermis and epidermis was rigidly clamped to the copper cylinder, water at 55 C was poured over the skin, and the excess water was removed by blotting. The clamps prevented lateral contraction of the heated tissue and the hot water facilitated subsequent removal of the epidermis. The conductivity determination was now made, the epidermis was then scraped off, and the determination repeated. As a further check, in certain experiments, a strip of intact epidermis was placed over the denuded dermis and the measurement repeated. The thickness of numerous pig epidermal strips was determined with a micrometer. The thickness was about $80 \pm 10 \mu$.

At least triplicate determinations were made on each of the four tissues of three different pigs (approximately 10 kg). The average values of the thermal conductivities obtained on each of these tissues are given in Table 4.

TABLE 4. *In vitro* thermal conductivities K of pig tissue, K given in (cal - cm)/(cm² - min - degrees C) units.

	Epidermis	Dermis	Fat	Muscle
K	0.036	0.054	0.021	0.064
	0.023	0.053	0.024	0.062
	0.032	0.051	0.023	0.073
K	0.03	0.053	0.023	0.066

In view of the thinness and uncertainty in the thickness of the pig epidermis, the wide variation in the epidermal thermal conductivity was to be expected. The data pertaining to the other tissues were considerably more reproducible.

It is of interest to compare some of these data with those of Breuer,⁸ who determined the respective thermal conductivities of both muscle and fat of cow, horse, pig, and dog. This investigator found that the conductivities of pig muscle and fat, expressed in the above units, were 0.060 and 0.021 respectively; furthermore essentially the same values were found for

the muscle and fat of the other three animals. In view of the excellent agreement between Breuer's value and the present one for pig muscle and fat, it is difficult to understand the value, 0.03, that Hardy and Soderstrom^{19,22} report for both cow muscle and fat. Unfortunately no description of their experimental method was given. In order to investigate this discrepancy, the thermal conductivity of beef muscle was redetermined and an average value of 0.057, which checks Breuer, was obtained.

In view of the numerous indeterminate factors (Section 17.3.1) which enter into the *in vivo* conduction of heat through pig skin, the *in vitro* thermal conductivities of these four tissues are not of themselves too useful. They do however serve as a baseline in the interpretation of certain experiments to be described.

OBSERVATIONS (*in vivo*) OF CALORIC UPTAKE OF PIG SKIN AND RISE IN TEMPERATURE AT DERMAL-FAT INTERFACE AS A FUNCTION OF BOTH TIME AND SKIN SURFACE TEMPERATURE

It was of interest to ascertain the caloric uptake of the skin when the epidermal surface was maintained at various temperature levels between 45 and 100 C. Numerous such experiments have been done and as was to be expected (see Section 17.3.1) the data were subject to wide variations and are extremely difficult to interpret in detail. Thus only a small fraction of these data will be reported and the variations to be expected will be indicated. During these experiments the temperature at the dermal-fat interface was also ascertained.

A pig under Nembutal anesthesia was clipped and shaved. The hypodermic needle thermocouple was introduced laterally into the dermal-fat interface. The skin temperature at the chosen site was determined and the automatic energy recording applicator was applied. Thus a continuous record of the caloric uptake of the skin at a predetermined epidermal surface temperature was obtained. The temperature at the dermal-fat interface was determined either intermittently with a Leeds & Northrup Type K2 potentiometer or continually with a General Electric photoelectric recording potentiometer.

Caloric uptake rate of pig skin: Typical caloric uptake data as a function of time and epidermal surface temperature are presented in Table 5.

The data given in Table 5 are a composite of at least three determinations on the lateral thoracic area of different pigs; five pigs in all were used. As

TABLE 5. A guide (± 30 per cent) to the caloric uptake of the skin as a function of time and surface temperature as determined by the automatic energy recording applicator.

Time interval in min	Skin surface temperature				
	45 C	50 C	55 C	60 C	65 C
Average caloric uptake rate in cal/min/cm ²					
0-0.2	6.0	9.5	12.0	15.0	17.0
0.2-0.4	2.2	3.7	4.9	6.9	8.4
0.4-0.6	1.8	2.8	3.8	5.6	6.7
0.6-0.8	1.7	2.5	3.1	5.1	5.9
0.8-1.0	1.6	2.3	2.7	4.7	5.4
1-1.5	1.5	2.0	2.6	4.3	4.8
1.5-2	1.4	1.8	2.4	4.1	4.5
2-3	1.2	1.6	2.3	3.7	4.2
3-5	1.1	1.5	2.1	3.2	3.8
5-7	1.0	1.4	2.0	2.8	3.5
7-10	0.9	1.3	1.9	2.5	3.2
Total caloric uptake in cal/cm ²					
0-1	2.7	4.2	5.3	7.5	8.7
0-5	7.5	10.7	14.3	21.8	25.2
0-10	12.2	17.4	24.0	34.9	42.7

was expected, in view of the numerous factors (Section 17.3.1) that determine the caloric uptake in a living animal, the experimental variations inherent to similar exposures were considerable. Thus these data served only as a rough guide (± 30 per cent) to the caloric uptake rate of pig skin.

It was noted that the average caloric uptake rate of pig skin during the first 0.2 minute was about sixfold greater than the average caloric uptake rate during the steady state period (7-10 minutes). This sixfold difference was due to the initial necessity of heat saturating the tissue and was primarily a heat capacity effect. After the first few minutes the skin tissue was essentially heat-saturated and the *in vivo* thermal conductivities of the various layers of pig skin primarily determined the caloric uptake rate. If the data given in Table 5 are plotted against time, the type of curve obtained will conform to that shown in Figure 2. A mathematical analysis of the general form of these curves based on equation (5) showed that during the first 2-3 minutes of the heat application, the skin can be considered as an infinite body with a ratio of thermal conductivity to heat capacity that is approximately the same as that computed from the *in vitro* determinations tabulated in Tables 4 and 5. This agreement was probably due to the fact that the ratio of the thermal conductivity to heat capacity (equation 6) was not nearly so sensitive to the indeterminate *in vivo* factors considered in detail in Section 17.3.1 as the individual quantities themselves.

TABLE 6. The time dependence of the dermal-fat interface temperature (± 20 per cent) during exposure of the surface of pig skin to predetermined temperatures.

Time in min	35.0 45	34.8 50	34.8 55	35.2 60	34.9 65	34.3 70	34.2 80	34.5 90	Surface temp C Initial During exposure
<i>Dermal-fat interface temperature C</i>									
0	34.7	34.5	34.6	35.0	34.7	34.2	34.4	34.8	
0.2	36	38	39	43	46	52	53	56	
0.5	38	43	45	46	52	62	65	66	
1.0	39.5	45	47	48	53	65.5	71	74	
1.5	40	47	48	47	53	66.5	72	77	
2.0	40.5	47	49	46	54	67	74	79	
3.0	41	47	48.5	45	56	67.5	75	79	
5.0	42	47	47.5	44.5	58	67.5	77		
7.0	42	46.5	47.5	47	58				
10.0	42	47	48	49	59				
<i>Average thickness of corium in mm</i>									
	2	2	2	2	2	2	2	2	Initial
	2	2.5	3.2	4.2	3	2.5	2	2	Termination of heat exposure
	0.06	0.1	0.09	0.10	0.16				<i>In vitro</i> thermal conductivity of dermis at termination of exposure

INTERFACE TEMPERATURE AT JUNCTION OF DERMIS AND FAT

The time dependence of the dermal-fat interface temperatures during the exposure of the skin surface to a predetermined temperature between 45 and 90 C is given in Table 6.

These values were a composite of at least two experimental determinations on two different pigs (four determinations in all). As in the case of the caloric uptake measurements, the variations in duplicate experiments were considerable, and these data only serve as a rough (± 20 per cent) guide to the time-temperature relationship at the dermal-fat interface. These data together with other experimental observations indicate the following.

1. The junction of the fibrous dermis and the subdermal fat in lateral thoracic area of a 10-kg pig lies about 2 mm below the skin surface. Ten-minute exposures to surface temperatures of 50 to 70 C increased significantly the thickness of the dermis. This increase in thickness was due to the accumulation of edema fluid in the dermis and the effect was maximal when the skin surface was maintained at about 60 C. Skin surface temperatures of 45 C or below do not activate the mechanism which gave rise to edema. Skin surface temperatures equal to, or greater than, 80 C denature the corium so rapidly that the mechanism by which edema fluid accumulated in the corium was destroyed.

2. Although the continual caloric uptake by the

skin tended to increase the dermal temperature, the appearance of relatively cool edema fluid tended to decrease it. At skin surface temperatures of 50 C and 70 C, these two effects nearly counterbalanced each other, and after the first minute of heat exposure the dermal-fat interface temperature remained essentially constant. With skin surface temperatures between 55 and 65 C the rapid appearance of a large amount of edema fluid more than compensated for caloric uptake, and the temperature at the interface between dermis and fat was temporarily lowered. This effect was maximal when the skin surface was maintained at about 60 C.

3. When the skin surface temperature was maintained at 45 C, and probably at all other temperatures that fail to cause edema, the dermis becomes "heat-saturated" after about 5 minutes of exposure. When edema fluid was produced, the time for dermal heat saturation was essentially indeterminate, but it apparently was greater than 10 minutes.

4. Histological examinations showed that complete primary injury to the dermis was obtained in all experiments where the skin surface temperature was maintained at 65° or higher. These limited (5) time-temperature-injury data at the dermal-fat interface tended to indicate a quantitative relationship very similar to that found for epidermal injury (see Section 17.7).

5. By making the reasonable assumption that the dermis is essentially "heat-saturated" at the end of a

10-minute heat exposure, the *in vitro* thermal conductivities of dermis can be computed by substituting the approximate caloric uptake (Table 5), dermal-fat interface and skin surface temperatures, and the final dermal thickness into equation (3) (Section 17.3.1); the neglect of the epidermal temperature drop introduced no appreciable error. Table 5 also shows the results of these calculations.

A comparison of these values with the experimentally determined *in vitro* values (Table 4) for pig dermis indicated that the presence of edema fluid increased the thermal conductivity of dermis two- to threefold. This increase in conductivity, however, was slightly more than compensated by the swelling of the dermis; an edematous dermis is thus a somewhat better heat barrier to the underlying tissues than normal dermis. A comparison of the *in vivo* thermal conductivity obtained at 45 C with the *in vitro* value of 0.053 (see Table 4) tends to indicate that intact circulation probably increased the effective thermal conductivity of dermis by about 15 per cent.

ESTIMATION OF TEMPERATURE CHANGES AT EPIDERMAL-DERMAL INTERFACE DURING EXPOSURE OF THE SKIN SURFACE TO HEAT

In view of the thinness ($\sim 80 \mu$) of the pig's epidermis, the experimental measurement of the time-temperature relationships at the epidermal-corium junction was not feasible.

There are certain facts, however, that allowed the estimation of this time-temperature relationship with a considerable degree of certainty. In view of the extreme thinness of epidermis, the temperature of the basal layer was largely determined by skin surface temperature, which was an accurately known quantity. This is most readily seen by solving heat conduction equation (3) for steady-state temperature of the basal epidermal layer. Of the four necessary experimental quantities, namely, skin surface temperature, epidermal thickness (about 80μ), epidermal thermal conductivity (Table 4), and caloric uptake of the skin at the requisite skin surface temperature (Table 5), only the last two were subject to a considerable variation (± 30 per cent). Fortunately, even variations of this magnitude resulted in uncertainties of less than 0.2 C in the steady-state temperature of the basal epidermal layer.

Basal Epidermal Temperatures When the Skin Surface is Immediately Brought to and Maintained at a Temperature between 45 C and 100 C. Before the

steady-state temperature is attained, the time-temperature relationship at this epidermal-dermal junction is given under these conditions to a good approximation by equation (6c) of Section 17.3.1, where γ has the following numerical value:

$$\gamma = 0.15$$

if the time t is expressed in *seconds*.

The numerical constant, 0.15, is not subject to the experimental uncertainties of the quantities requisite to computation by equation (6a), since it can be quite accurately determined empirically from the temperature-time-epidermal injury data (see Section 17.6.5 for details). An identical value for γ can also be directly computed by substituting into equation (6c) the experimentally determined values for heat capacity, thermal conductivity, and thickness of epidermis, and by assuming an epidermal density of 0.8 g/cc (a most reasonable value). In view of the two completely independent methods, one of which was *in vivo* and the other *in vitro*, considerable confidence could be placed in the adaptation of the infinite body picture (see Section 17.3.1) to the solution of the time-temperature relationship at the epidermal-dermal junction during the unsteady state period of heat flow.

The computation of the temperature of the basal cell layer of the epidermis as a function of both time and skin surface temperature is given in Table 7A.

TABLE 7A. The computed time-temperature relationships for the epidermal-dermal interface when the skin surface is immediately brought to and maintained at a specific temperature.

Time in seconds	Surface temperature, C				
	45	55	65	80	100
	Temperature at basal epidermal layer* C				
0	35.0	35.0	35.0	35.0	35.0
0.01				36.3	37.0
0.02			38.9	40.9	43.4
0.05		41.8	45.2	50.3	57.1
0.1	40.1	45.2	50.3	57.9	68.2
0.2	41.3	47.6	53.9	63.3	75.9
0.5	42.7	50.4	58.1	69.6	85.1
1.0	43.3	51.6	60.0	72.4	89.1
2.0	43.8	52.6	61.4	74.6	92.3
5	44.2	53.5	62.7	76.6	95.1
10	44.5	53.9	63.4	77.6	96.6
30	44.7	54.4	64.1	78.6	98.0
60 (1 min)	44.8	54.6	64.4	79.0	98.6
120 (2 min)	44.9	54.9	64.5	79.4	99.2
300 (5 min)	44.9	54.9	64.7	79.5	99.3
600 (10 min)	44.9	54.9	64.8	79.7	99.6
Steady state†	44.8	54.5	64.2		

* Computed by equation (6c) and experimental data of Section 3.2.

† Computed by equation (3) and experimental data of Section 3.2.

TABLE 7B. The computed time-temperature relationships for the epidermal-dermal interface when an entire animal (~ 30 cm in diameter) is surrounded by an envelope of ambient and radiant heat that results from a constant temperature source.

Time in seconds	Circumambient temperature, C				
	80	100	125	150	175
	Heat transfer coefficient H^* in cal/cm ² /min per C				
	0.015	0.019	0.021	0.024	0.026
	Temperature at basal epidermal layer, C ^{†,‡}				
0	35	35	35	35	35
10	37	39	40.5	44	46
20				46.5	49
30	38.5	41.5	44	49	52
40				51	54.5
50	39.5	43.5	46.5	53	57
70	40	44	48	56	60
100	41	45.5	50	59	64
130	42	47	52	61	
160	42.5	48.5	54.5	63	
200	43	50	56	65	
300	45	52.5	59		
400	46	55	63		
500	47				
600	48				
800	50				
1,000	50.5				
1,200	51				

* In order to make these data directly comparable to the experimental investigations of Section 17.9, the radiant contribution to H was computed by using a source temperature 20 per cent in excess of the air temperature.

† Computed by means of equations (5), (6), (6a), and (6b) and experimental data of Section 17.3.2.

‡ Because of both the thinness of the epidermis and the slow rate of heat transport to the skin, there is no appreciable difference between these temperatures and those of the skin surface after the first 20 seconds of heat exposure.

The data given in Table 7A show that there was a rapid rise in the temperature of the basal epidermal layer when the skin surface was immediately brought to and maintained at a specified constant temperature. A comparison of the unsteady-state data computed from equation (6c) with the steady-state data obtained by means of equation (3) showed that the epidermis under the above conditions became essentially "heat-saturated" after a heat exposure of 0.5- to 1.0-minute duration.

Actually, only the unsteady-state time-temperature relationship as given by equation (6c) need be considered to elucidate the irreversible epidermal injury threshold data of Section 17.6.5; since these experimental time-temperature-epidermal injury relationships were such that for all skin surface temperatures above 50 C the epidermis never reached heat saturation, and for all temperatures below 50 C the difference between the steady-state basal epidermal

temperature and the skin surface temperature was trivial.

It must be re-emphasized that these data apply only to situations in which the heat transfer coefficient H from the temperature source to the skin surface is infinite.^c In all cases where H is finite an analysis similar to that given below is required.

Basal Epidermal Temperatures When the Entire Animal Is Surrounded by an Envelope of Ambient and Radiant Heat between 80 and 175 C. In the previous section, the time-temperature relationships at the epidermal-dermal junction depended only upon the rate of heat transfer through the skin and the constant temperature of the heat source. To this must now be added the slow rate at which heat is transported from the heat source to the skin surface via air conduction, air convection, and infrared radiation. The mathematical solution of this problem is given by equation (6), where the only quantity that requires further consideration is H , the heat transfer coefficient from the heat source to the skin surface. This quantity is readily computed through the substitution of equation (1), heat transfer by convection, and equation (2), heat transfer by radiation, into equation (5). The numerical values of the heat transfer coefficient which were obtained at certain source or air temperatures are shown in Table 7B. A comparison of the numerical values of H , 0.015 to 0.026 calorie per square centimeter per minute per C, with epidermal thermal conductance K/L (Table 4) numerically equal to 4 in the same units, indicates the slow rate at which ambient and radiant heat is transferred to the skin surface as compared with the rate this heat flows through the epidermis.

Table 7B also gives the estimated temperature of the basal epidermal cell layer as function of source or air temperature as calculated by means of equation (6). These data show the extreme slowness of temperature rise at this epidermal-dermal junction. In fact, under these conditions, the epidermal temperature even after a heat exposure of 15 minutes is far lower than the temperature of the heat source, and one would expect an animal to succumb to hyperthermia long before the temperature of the skin approached that of the air.

Although the data for the time-temperature relationship at the skin surface are not given, they can be

^c Under the experimental conditions to be described in Section 17.6 (hot water experiments), H is not infinite³⁵ but rather about 10^5 cal/cm²/min per C. In these computations the substitution of ∞ for 10^5 is of no significance.

readily computed by putting L (the thickness of the epidermis) equal to zero in equation (6). If this be done, it will be found that, except for the first 20 seconds of heat exposure, the skin surface temperature is not significantly different from the values recorded in Table 7B for the basal epidermal temperature. This is due to the fact that heat transfer to the skin is the controlling factor. Thus, these data can also be taken as the temperature of the skin surface as a function of time.

A comparison of Tables 7A and 7B indicates the importance to the epidermal time-temperature relationships of the mode of imparting heat to skin surface. Thus, for a given source temperature, a mechanism that enables the surface temperature to be immediately brought to and maintained at the source temperature has, on a time basis, at least a thousand times greater injury propensity to epidermis than a heat source which raises the skin temperature by means of radiation, conduction, and convection of relatively immobile air. (See Section 17.9.2 under "Measurement of Heat Transfer.")

17.3.3 Summary

The various physical factors which determine the transfer of heat energy to and through the skin and the temperatures attained thereby have been defined and discussed.

A general theory of heat flow through the epidermis is developed.

Experimental observations pertaining to the rate at which heat energy is taken up by the skin during surface exposures of varying intensity and the subsurface thermal gradients established therein have been presented.

The time-temperature relationship at the dermal-epidermal junction is computed under two greatly different experimental conditions: (1) when the skin surface temperature is immediately brought to and maintained at the temperature of the heat source, and (2) when the entire skin surface is exposed to a specified circumambient and circumradiant temperature. These data indicate the extreme importance of the mode of applying heat to the skin surface to the time-temperature relationships within the epidermis.

17.4 EFFECTS OF INHALED HEAT

It was inferred from the results of the pilot experiments (Section 17.2.5) that, so far as rapid neutralization of enemy personnel by flame thrower attack

is concerned, the effects of heat on the surface of the body are probably of greater importance than are its effects on the air passages and lungs. The implication of this assumption is too great to accept at face value the small amount of evidence provided by the pilot experiments.

A search of the literature failed to disclose any reliable information concerning the effects on the lungs and air passages of inhaled heat or the circumstances in which thermal injuries of the respiratory tract may be sustained. The following investigation was accordingly undertaken.³⁷

17.4.1 Experimental Procedure

In order to study the effects of heat on the respiratory tract independently of the secondary changes that might result from concomitant burning of the skin, dogs were caused to breathe hot air which was conducted directly to the trachea through an insulated transoral cannula.

In some experiments heated air was pumped directly into the air passages and in others it was inhaled by the respiratory efforts of the animal. The inner end of the cannula extended below the vocal folds of the larynx. Three types of inhalation experiments were performed. In the first the animals breathed room atmosphere heated to temperatures as high as 500 C in an oven. In the second, flame from a blast burner at temperatures estimated to be in the vicinity of 1000 C was directed into the external end of the cannula. In the third, a mixture of live steam and air was breathed from a generator (see Figure 5). All experiments were conducted under anesthesia induced by the intravenous or intraperitoneal injection of sodium pentobarbital.

The external temperature of the air available for respiration in each type of experiment was measured either by a thermometer or a platinum-rhodium thermocouple. Thermocouples (40 gauge copper-constantan) were installed in the airway, one at the laryngeal end of the transoral cannula and the other at or near the bifurcation of the trachea, to measure the rate at which the inhaled air was cooled. Leads from these thermocouples were connected with a Mohl galvanometer having a period of 0.2 second. The excursions of the galvanometer were observed directly and recorded manually.

17.4.2 Rate of Cooling of Inhaled Air

When the superheated air was inhaled, the temperature recorded by both the laryngeal and the

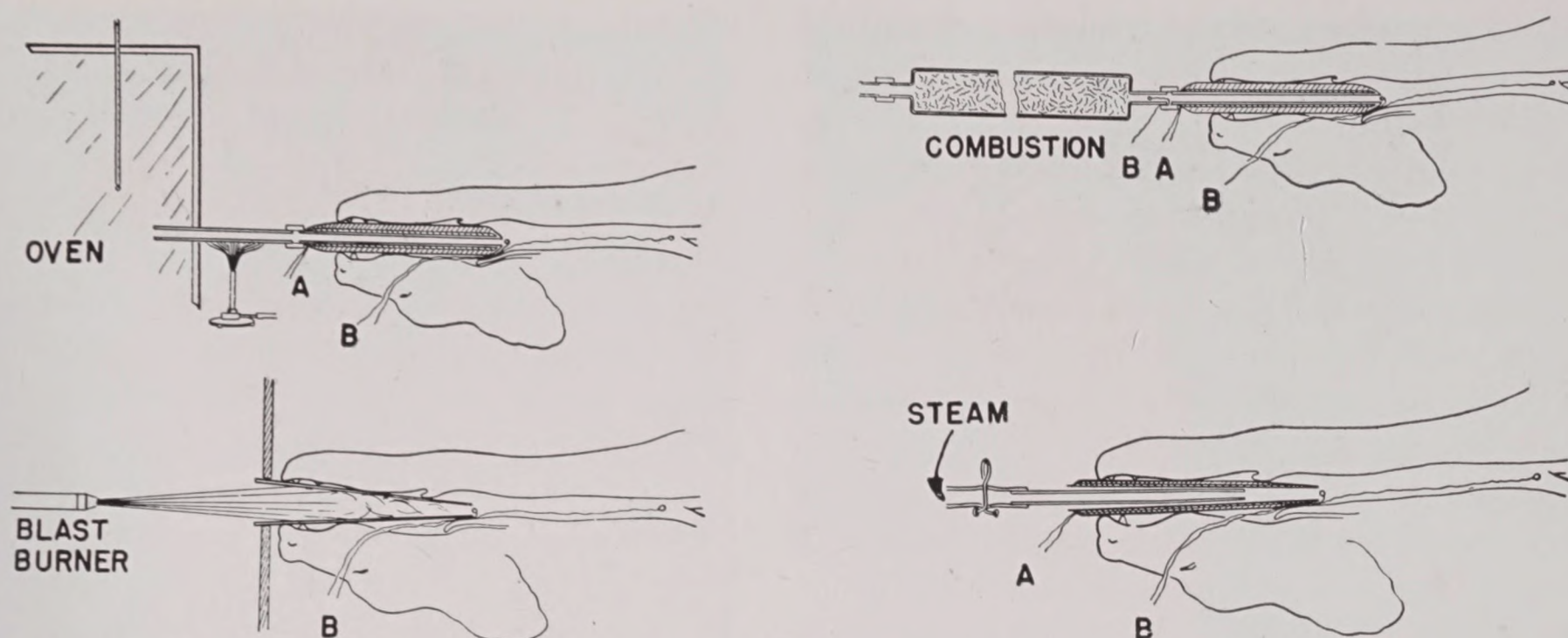


FIGURE 5. Experimental procedure used to investigate effects of inhaled heat on air passages and lungs. In all instances, insulated cannula conveyed hot air, flame, or steam from outside to animal's larynx. Position of intra-laryngeal and deep tracheal thermocouples is shown. *Top left view:* Animal breathed room temperature heated in oven to 350 C. *Top right view:* Room temperature was pumped into animal's lungs from combustion oven which was heated to 500 C. *Bottom left view:* Flame and combustion products of blast burner were projected into cannula during each inspiration. *Bottom right view:* A 400 ml blast of mixture of live steam and air was released into transoral cannula at the beginning of each inspiratory effort. Results of these experiments are shown in Table 8.

TABLE 8. Results of experiments in breathing of hot air.

Kind of atmosphere breathed	No.	Animal No.	Original pre-inspiratory temp of air (C) (approximate)	No. of breaths	Max temperature recorded (C)		Recovery period (hours)	Site and severity of injury		
					Laryngeal cannula	Lower trachea		Upper trachea	Lower trachea	Lungs
Air from drying oven. See Fig. 5A	1	423	350	46	182	...	19	Mild	None	None
	2	420	350	52	180	...	19	Mild	None	None
	3	391	350	103	159	...	30	Mild	None	None
	4	390	350	106	175	...	Not killed	(Complete clinical recovery — no autopsy)		
Air from combustion oven. See Fig. 5B	5	392	500	60	267	...	4	Mild	None	None
	6	432	500	44	327	50	7	Moderate	None	None
	7	426	500	22	291	...	24	Mild	None	None
	8	431	...	17	...	135	7	Moderate	Mild	None
Flame from blast burner. See Fig. 5C	9	433	...	10	327	51	8	Severe	Moderate	Mild
	10	454	...	16	540	100	11	Severe	Mild	None
	11	455	...	24	550	65	24	Moderate	Mild	None
	12	405	...	14	510	64	Not killed	(Complete clinical recovery — no autopsy)		
Steam from generator. See Fig. 5D	13	456	Over 100	27	106	59	6	Moderate	Mild	None
	14	519	Over 100	18	98	79	7	Severe	Moderate	None
	15	481	Over 100	20	94	53	10	Severe	Severe	Severe
	16	475	Over 100	16	99	94	10	Severe	Severe	Severe
	17	524	Over 100	10	...	90	24	Severe	Severe	Moderate
	18	522	Over 100	12	...	75	48	Severe	Severe	Mild

tracheal thermocouples rose throughout inspiration and fell during expiration. In each situation the highest point in the temperature curve was reached at or near the end of inspiration. The inhaled gas lost most of its heat before reaching the lungs. When the inhaled gases were relatively dry, the intratracheal temperature rose to a sharp peak and fell away

rapidly during expiration. When steam was inhaled, the curve described a plateau rather than a peak, probably because of the condensation of hot water on the thermocouple. The results of these experiments are shown in Table 8.

When air heated to between 350 and 500 C was inhaled, the temperature fell to about half of its ex-

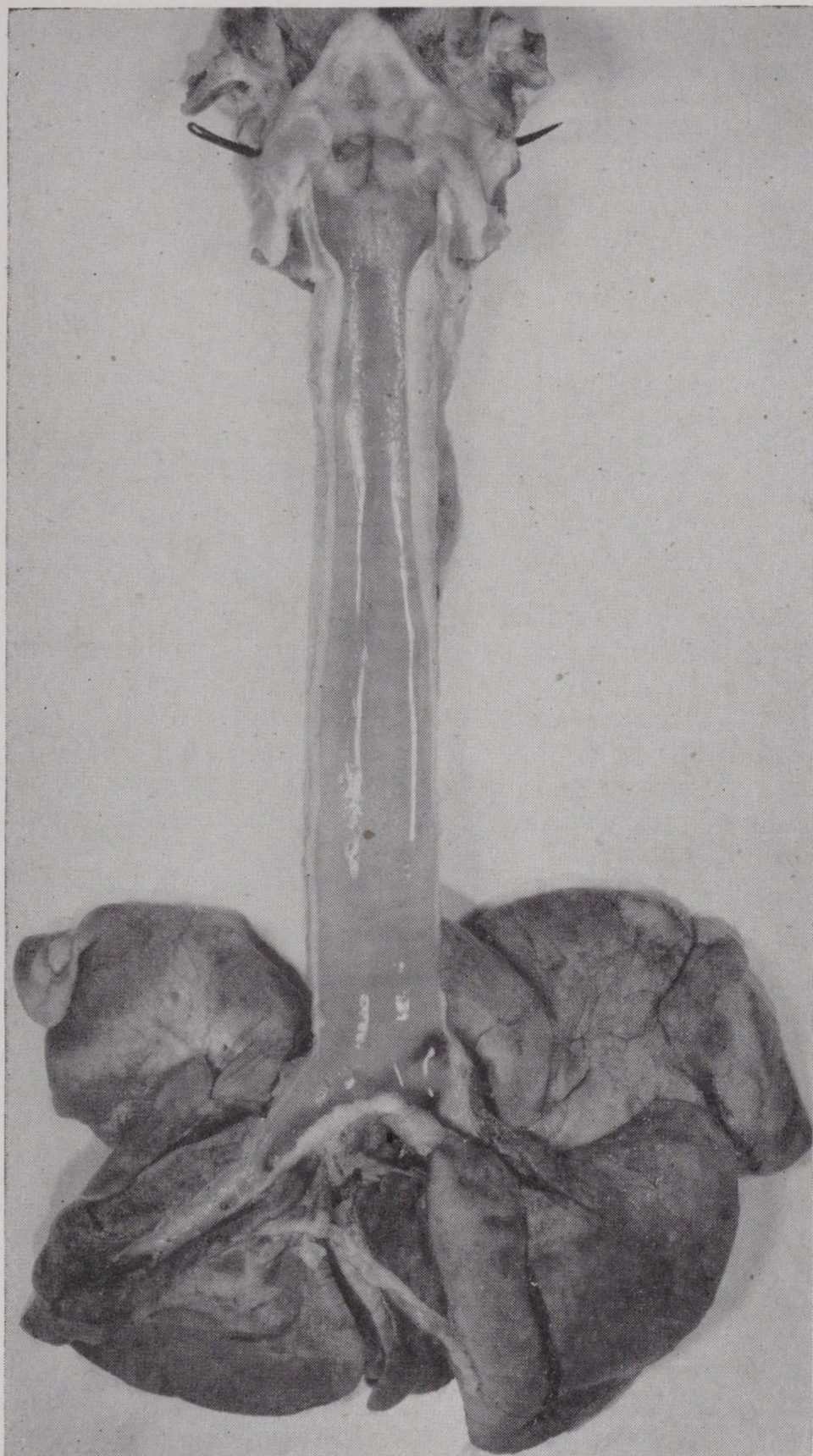


FIGURE 6. Thermal laryngitis and tracheitis without pulmonary injury. Photograph of respiratory tract of dog 24 hours after inhalation of flame. Sufficient heat had been conducted through wall of cannula to cause mild degree of laryngeal edema which may be recognized by bilateral olive-shaped mucosal protrusions from ventricular recesses. There was extensive destruction of mucosa of upper trachea, diminishing rapidly to mild catarrhal inflammation in lower third. No abnormality of bronchi or lungs of this animal was recognized.

ternal level by the time it reached the larynx, despite the fact that it was conducted through the mouth by means of an insulated cannula. By the time it had reached the bifurcation of the trachea, the temperature had dropped to approximately 50 C. Flame and combustion products of a blast burner directed into the external end of the transoral cannula were delivered to the larynx at temperatures between 300 and 550 C. The highest recording at the bifurcation

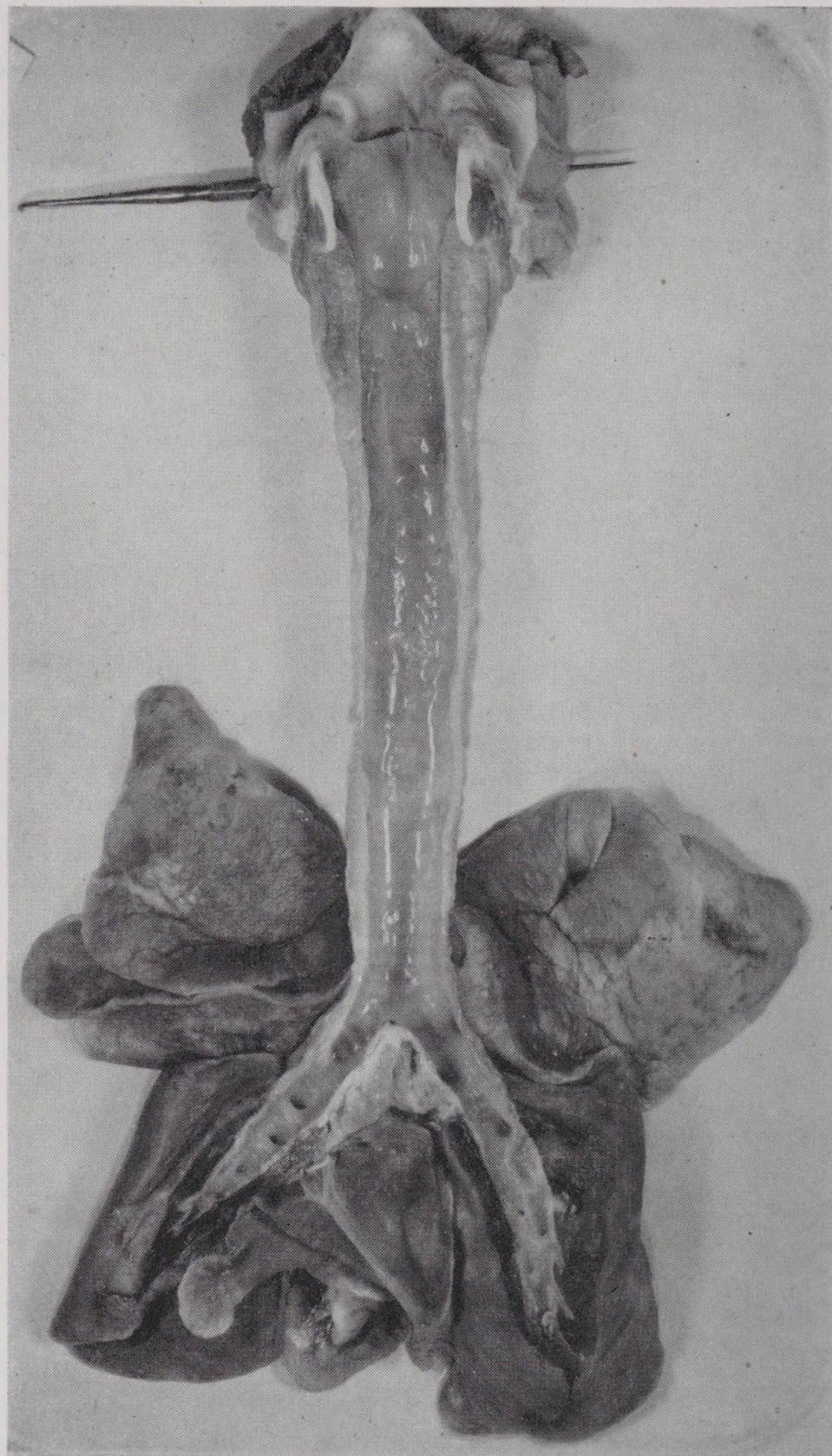


FIGURE 7. Thermal tracheitis and pneumonitis. Photograph of respiratory tract of dog 10 hours after inhalation of steam, showing severe tracheobronchitis with dilatation of bronchi. There is central hemorrhagic pneumonitis with generalized pulmonary edema and hyperemia.

of the trachea in such experiments was 135 C. When a mixture of live steam and air was inhaled, the inspiratory peaks recorded at the laryngeal opening of the cannula ranged between 94 and 106 C and those by the deep tracheal thermocouple, between 53 and 94 C.

17.4.3

Effects on Animals

The mildest thermal exposure used in the inhalation experiments was more than sufficient to cause severe injury to the skin. Every animal included in Table 8 would have sustained severe cutaneous injury if the skin had been exposed for more than a few

seconds to such temperatures. Circumambient air temperatures as low as 300 C produce severe injury of unprotected skin within a few seconds. Mixtures of steam and air at 100 C destroy epidermis even more quickly.

Early in the investigation it was found that if animals were to survive the inhalation experiments long enough to develop reactive changes in the lower air passages it was necessary to protect the larynx. Otherwise they died prematurely of asphyxia due to laryngeal edema. For this reason the transoral cannula was inserted well below the glottic folds.

Primary thermal injury of the lungs occurred in none of the 7 animals that breathed hot air, in only 1 of the 5 animals that inhaled flame from a blast burner, and in 4 of the 6 animals that inhaled live steam. In the remaining animals thermal injury to the respiratory tract was confined to the upper air passages. In no instance did an animal die as a result of thermal injury of the lungs within the first 24 hours. All animals that sustained thermal injuries of the respiratory tract would, under nonexperimental conditions, have received severe cutaneous burns.

Mucosal necrosis with desquamation of surface epithelium occurred in all instances where the blast of hot atmosphere first struck the lower portion of the larynx and the upper portion of the trachea. In the case of hot air the injury was usually localized and represented by shallow ulceration associated with catarrhal inflammation of the upper third of the trachea (Figure 6). Inhalation of flame or steam led to extensive destruction of the trachea with edema of the peritracheal areolar tissue of the neck and mediastinum and detachment of large casts of necrotic mucous membrane, which were either expelled by coughing or subsequently inhaled into the lower portions of the respiratory tract (Figure 7).

The portions of the lungs most vulnerable to injury were the centrally located alveolar ducts and their communicating alveoli (Figure 8). Atmosphere not hot enough to damage the mucosa of the large bronchi or the alveoli of the more peripheral portions of the lungs was in some instances capable of causing central pulmonary edema and both intra-alveolar and interstitial hemorrhage. After more severe exposures the lungs became diffusely edematous and hemorrhagic. Focal patches of atelectasis and emphysema were observed and in some instances were obviously due to aspiration of mucus or mucosal debris. Bronchopneumonia was commonly observed in animals that had received tracheal burns. It ap-

peared that, regardless of the mildness of the primary thermal injury of the lungs, if the inhaled air was hot enough to damage the trachea it usually predisposed the animal to pneumonia.

17.4.4

Discussion

It was apparent from the foregoing observations that air hot enough to burn the skin can be inhaled without causing damage to the trachea or lungs and that if the temperature of the air is high enough to damage the respiratory passages it will inevitably have caused burning of the surface of the body.

This observation seemed paradoxical in view of the fact that the mucosa of the air passages is much thinner than the skin and should therefore be more vulnerable to thermal injury. The explanation of the experimental findings lies in the fact that the quantity of heat that can be stored in the volume of gas that constitutes a breath is remarkably small. At any given air temperature the number of calories that can be transferred to the respiratory tract incident to the inhalation of a breath of hot air is limited by the volume of that breath, whereas convection currents are capable of bringing a practically unlimited volume of hot air in contact with the skin. An infinitely greater caloric transfer can occur for each unit of surface exposed.

Not only is the amount of heat energy available for transfer to the skin greater than that which is available for transfer to the respiratory membranes but also there are important time differences between cutaneous and respiratory exposures. In the case of the skin the exposure is virtually continuous, whereas the lining of the air passages is exposed intermittently as each new breath is inhaled.

An instructive illustration is provided by calculating the potential heat transfer to the respiratory tract that might occur if air were inhaled at 142 C. Let it be assumed that the amount inhaled with each breath would be sufficient to increase the pulmonary volume by 500 ml, that the air was dry when inhaled and saturated with moisture when exhaled, and that it was cooled to body temperature by the time it left the body. Approximately 13 cal of heat energy could be released within the body by cooling of one such breath from 142 to 38 C. Theoretically this amount of heat would be sufficient to raise the temperature of 1 g of tissue by approximately 13 degrees, providing none of it was carried away by the blood circulating in the subsurface capillaries. Actually no change in the net temperature of the respiratory



FIGURE 8. Primary thermal pneumonitis. Photomicrograph of lower lobe of dog's lung 24 hours after inhalation of steam. Although there was severe tracheitis, primary and secondary bronchi showed remarkably little change. Evidence of pulmonary injury was confined largely to the central portions of lobes and consisted of hyperemia, edema, and partial atelectasis.

tract would occur in such circumstances because the gain of 13 cal would be offset by a loss of 13 cal incident to the evaporation of the 23 mg of water that would be required to saturate that amount of dry air.

This is not to imply that the inhalation of air heated to 142 C would be necessarily harmless. Desiccation would probably occur near the portal of entry even though there were no net change in the temperature of the respiratory tract as a whole. The calculation serves to emphasize how important the heat capacity of the inhaled gas is in relation to the problem of thermal injury of the lungs. A rise in tissue temperature is prerequisite to the occurrence of thermal injury and the amount that the tissue tem-

perature is raised incident to any given exposure will depend in part on the magnitude of temperature differential and in part on the amount of heat energy that the inhaled gas is capable of storing.

A more important attribute of an inhaled hot gas than its temperature in relation to its capacity to cause thermal injury is its water content. When steam or a mixture of steam and air comes in contact with a cool surface such as the skin or the lining of the respiratory tract, water is condensed on the surface with liberation of a relatively large amount of heat.

Thus the cooling of a 500-ml mixture of equal parts of steam and air from 125 to 38 C would lead

to the condensation of about 300 mg of water. The heat energy liberated by this amount would be in the neighborhood of 175 cal. There is little doubt but that the sudden liberation of 175 cal to the lining of the air passages or on the surface of the skin would be capable of causing some injury.

17.4.5 Summary

It was apparent from these experiments that thermal injury of the lungs is probably a negligible factor in the causation of disability or death incident to exposure to conflagrations such as might result from flame thrower action. A thermal exposure of sufficient intensity to cause direct injury of the lungs was more than sufficient not only to cause extensive burning of unprotected skin but also to result in rapidly fatal obstructive edema of the glottis. In the case of externally unburned or mildly burned casualties of a flame attack it can be assumed that no significant thermal injuries of the respiratory tract have been sustained.

17.5 COMPARISON OF PORCINE AND HUMAN SKIN

The original choice of the pig as a suitable subject for this investigation was based on the fact that no other readily available animal has skin that bears so close an anatomical resemblance to that of man.

A comparison of the structural characteristics of porcine and human skin at this point seems desirable in view of the extent to which the pig was used in experiments designed to provide information regarding (1) the reciprocal relationship of time to temperature in the production of cutaneous injuries in man, and (2) the local and systemic disturbances in man which cutaneous hyperthermia may be capable of causing.

Like that of man the surface of the pig's body is covered by three layers of tissue. Progressing from outside in, these are the epidermis comprising stratified epithelial cells, the dermis comprising fibrous connective tissue, the hypodermis comprising fibrous connective tissue, and the hypodermis comprising fibroadipose tissue (see Figures 9 and 10).

17.5.1 Epidermis

The epidermis of the pig varies in thickness, the average over the lateral body surface of immature animals (8-12 kg) being approximately 0.1 mm, which is slightly less than that from corresponding areas of adult human subjects. As with man there are irregularities in contour of both the upper and

lower surfaces of the epidermis, those on the upper surface being due to an intricate system of intercommunicating linear depressions and those on the lower surface corresponding to the dermal papillae over which it is moulded. The hairs penetrating the epidermis of the pig are thicker and more numerous than those of man.

Microscopic appearance of epidermis: Like that of man the outermost zone of epidermis or stratum corneum of the pig consists of several loosely connected layers of the desiccated and intensely basophilic remains of keratinized epithelial cells.

The second or granular layer is thin and consists of several layers of dead or dying squamous cells, the acidophilic cytoplasm of which contains many fine, deeply basophilic kerato-hyaline granules. Many of these cells have lost their nucleuses. Others contain shrunken hyperchromatic nucleuses or Feulgen negative nuclear ghosts.

The third zone is comprised of several layers of aging squamous cells which no longer have any direct cytoplasmic attachment to the dermis. The cytoplasm is dense, deeply acidophilic, and appears desiccated. The cells are so closely packed that neither intercellular bridges nor spaces can be recognized. Many of the nuclei are relatively small and more densely packed with chromatin granules than those of the deeper cells.

The fourth zone consists of cells in transition between the squamous and the basal cell layer. The transitional cells are large and polyhedral and many of them still have an attenuated footlike cytoplasmic attachment to the dermis. It is in this zone that intercellular bridges of tonofibrils are most readily visualized. The cytoplasm is moderately basophilic. The cell outlines are distinct and the intercellular spaces are clearly defined. The nuclei are larger and rounder than those of the more superficial cells and contain several coarse and many fine granules of chromatin.

The fifth zone is comprised of the basal cells, which, except for their cuboidal or columnar shape and their palisadelike arrangement on the dermis, are essentially similar to the overlying transitional cells. Projecting from the inferior surface of the basal epidermal cells of the pig are many robust tonofibrils which appear to be embedded in the dense felt work of fine collagen fibrils that comprise the superficial zone of dermis. No such fibrillar anchorage of epidermis to dermis can be seen in human skin (see Figures 19 and 20).



FIGURE 9

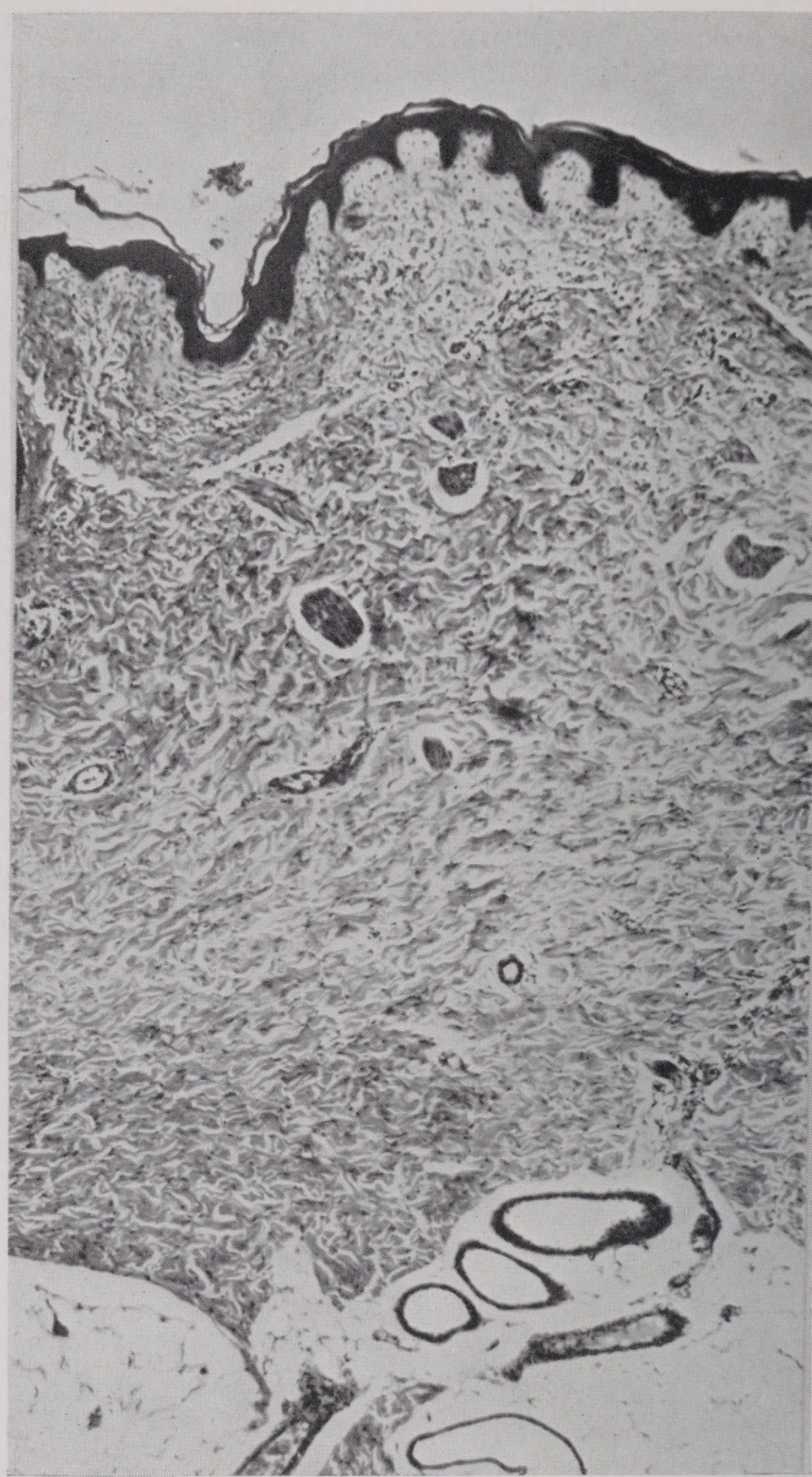


FIGURE 10

Appearance of porcine (Figure 9) and human (Figure 10) skin under low magnification, stained with phloxine-methylene blue. Sections are representative of lateral thoracic region of pig and lateral abdominal region of man. Epidermis is slightly thicker in man, and dermal papillae are broader in pig. Collagenous bundles in dermis of pig are heavier than those in man. Glands shown in hypodermis of pig do not secrete sweat.

The microscopic appearance of the epidermis of both man and pig suggests that there is a progressive loss of intracellular water as the epithelial cells grow older and move away from the dermis. The nearer the surface is approached, the more desiccated the cells appear. The entire stratum corneum and most of the cells of the granular layer appear to be dead and incapable of vital reaction (see Figures 17 and 18).

17.5.2

Dermis

The dermis covering the lateral body surface of immature pigs measures between 1.0 and 2.0 mm in

thickness and is generally more compact than that of man. In both pig and man the superficial portion of the dermis comprising the papillary layer or corium is characteristically a soft, thin, loosely arranged felt work of delicate collagen fibrils in which there appears to be an abundant amount of interstitial fluid. In man it is readily distinguishable from the thick underlying reticular layer, which is comprised of robust and closely interwoven bundles of collagen fibrils. Elastic fibrils are more numerous in human than they are in porcine skin. On the lateral body surface of the pig the corium tends to be thinner and less well defined than it is in man and in places is

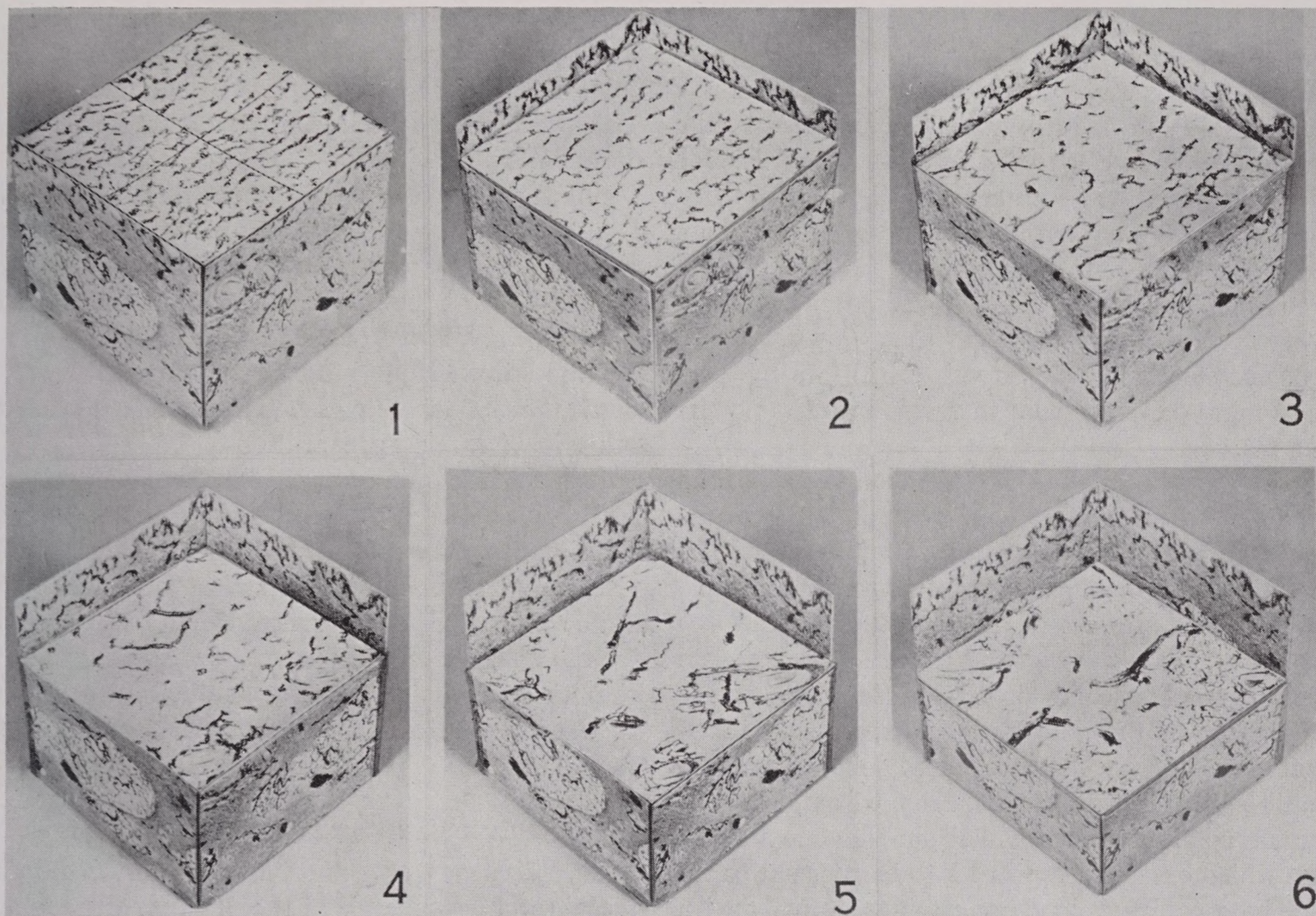


FIGURE 11. Series of composite photomicrographs showing vascularization of block of hyperemic porcine skin which measured 2x2x2 mm. Series of thick (50μ) benzidine-treated horizontal and vertical sections were mounted in such a way as to show distribution of veins, arteries, and capillaries at various levels beneath surface. No. 1 shows capillary plexus lying in most superficial (50μ) portion of dermis. No. 6 shows vessels in most superficial layer of adipose tissue of hypodermis.

only slightly less compact than the reticular zone. The deeper portion of the reticular connective tissue sends trabecular extensions into the underlying adipose hypodermis.

BLOOD VESSELS OF PORCINE SKIN

It was observed in ordinary histological preparations that the appearance of the capillaries in the dermal papillae of the body skin of the pig is similar to that in corresponding regions of man. In recognition of the fact that it is difficult or impossible to get an accurate impression of so complicated a structure as a capillary network by two-dimensional visualization, a modification of the Pickworth technique¹⁰ was employed in order that the dermal blood vessels could be studied in three dimensions.

Maximum cutaneous hyperemia was induced beneath a circumscribed area of the lateral body surface of the pig by exposure to water at 50 C for 20 minutes. After such an exposure the erythrocytes

were so densely packed in the distended capillaries that there was practically no loss of blood from them when the skin was excised. Skin and subcutaneous tissue treated in this way was excised to a depth of 8 mm, fixed in 10 per cent formalin, cut in thick sections, and treated with benzidine.

The benzidine combined with the hemoglobin to impart a dark blue color to the contents of the engorged vessels. After skin treated in this manner was cleared, a three-dimensional study of its blood vessels could be made by use of a binocular microscope.

The appearance of the dermal vessels of porcine skin at various levels below the surface is shown in Figure 11. To prepare this illustration a block of benzidine-treated skin was cut serially and parallel to the surface in sections measuring 50μ in thickness. Another block of the same skin was cut serially and at right angles to the surface. Photographs were made of both series and the prints were mounted in such a manner as to orient the horizontal sections in

relation to the depth below the surface that each represented.

The epidermis was removed from the surface of the block of skin shown in Figure 11. The excised skin was not clamped prior to fixation and postexcisional contraction resulted in an accentuation both in the height of the dermal papillae and also in the thickness of the dermis. It may be seen that the fibrous dermis including the papillae measures approximately 2 mm in thickness and that broad septa of fibrous connective tissue extend down from the dermis at more or less regular intervals into the underlying fat.

In approaching the surface the blood vessels to the skin followed an oblique course through the hypodermis and after reaching the lower layer of the fibrous dermis branched horizontally with multiple intervenal and interarterial anastomoses. From these first approximately horizontal plexuses originated a series of broad vascular loops that penetrated to the mid-portion of the dermis. Interarterial and intervenal anastomoses between these loops served to establish a mid-dermal plexus. From this mid-dermal plexus originated numerous hairpin-shaped capillary loops which extended upward into the dermal papillae. These capillary loops anastomosed freely with one another and constituted the most superficial or papillary plexus. It was apparent that capillary communications between the arterioles and venules occurred at different levels. Some followed a course that brought them to within a few microns of the basal epithelial cells over the tips of the papillae. Still others followed an almost horizontal course to establish communications between the arterioles and

venules of the intermediate plexus. At all levels through the dermis there were numerous vascular communications with the mantlelike meshwork of capillaries that surrounded the hair follicles and dermal glands.

As may be seen in Figure 11 the number, size, distribution, and communications of the dermal blood vessels of the pig are remarkably similar to those described by both Lewis³³ and Spalteholz⁴³ in human skin. The similarity of blood vessels in human and porcine skin was found to be so great that it was with difficulty that one could be distinguished from the other in Pickworth preparations.

It is not intended to imply that the anatomical resemblance between the vessels of human and porcine skin implies an equal degree of functional similarity. Certainly the vascularization of both indicates that ample and similar mechanical facilities exist either for the transfer of body heat to the surface to facilitate its dissipation, or for the conduct of surface heat to the interior to raise the internal temperature of the body.

SWEAT GLANDS AND SWEATING

Several types of glands are encountered in the dermis and hypodermis of the pig and, although one of them bears some resemblance to the sudoriferous glands of human skin, it does not secrete a significant amount of sweat.

The fact that the pig does not sweat was verified by a series of experiments in which the water loss from the skin of living pigs was measured at various environmental temperatures, with and without the administration of pilocarpine (see Table 9).

TABLE 9. Rate of water loss from surface of human and porcine skin. Amount of water loss determined by accretion in weight of $\text{Mg}(\text{ClO}_4)_2$ contained in base of weighing bottle during the time that the neck of the bottle was held in contact with the skin.

	Water uptake ($\text{mg}/\text{cm}^2/\text{min}$) during a period of 10 minutes							
	Temp 21 C — Humidity 30–40%				Temp 36 C — Humidity 30–40%			
	No. of tests	Min	Max	Mean	No. of tests	Min	Max	Mean
Dead pig (lateral thoracic region)	4	0.016	0.026	0.019	4	0.023	0.031	0.027
Live pig (lateral thoracic region)								
Without pilocarpine	5	0.016	0.020	0.021	6	0.020	0.032	0.028
Live pig (lateral thigh)								
Without pilocarpine	.				4	0.018	0.028	0.024
With pilocarpine*	.				4	0.021	0.030	0.027
(1 mg/kg bwt)								
Live man (forearm)								
Subject #1 (A.R.)	1			0.027	1			0.180
Without pilocarpine								
Subject #2 (A.M.)	2	0.028	0.038	0.033	2	0.280	0.360	0.320
Without pilocarpine								

* Iodine color test negative.

It was found that the water loss from the skin of a live pig does not differ significantly from that of one that is dead. In a cool environment the water loss per square centimeter per minute is approximately the same in man and pig. At higher environmental temperatures, the rate of water loss from human skin is tremendously augmented, whereas the corresponding increase in water loss from the skin of a pig is relatively small and is due to more rapid evaporation of tissue water rather than to sweating.

17.5.3 Summary

So far as can be judged by anatomic criteria the pig should be a suitable experimental subject from which to derive certain types of information regarding the effects of heat on human skin. Its various layers are of comparable thickness and structure. Its blood vessels are similar in size, number, and distribution. As will be shown later in Sections 17.6 and 17.7, its susceptibility and reactions to control episodes of hyperthermia are remarkably similar.

Since a pig does not sweat, allowance should be made for the inability of porcine skin to lose heat through the vaporization of moisture derived from sweating. The significance of heat loss through vaporization of moisture in respect to cutaneous burning will be discussed in greater detail in Section 17.9.

17.6 RECIPROCAL RELATIONSHIPS OF TIME AND TEMPERATURE^{3b}

The most direct mechanism by which exposure of the body surface to excessive heat results in injury is the transfer of heat energy to the skin at so rapid a rate that its temperature is raised to a level incompatible with cellular survival. Such localized thermal injuries are commonly referred to as burns. Although it is common knowledge that there is an inverse relationship between temperature and the amount of time required to produce a burn, there is remarkably little precise information regarding the rate at which burning occurs at any given temperature.

Because of the experimental difficulties inherent in the making of accurate measurements of either the time or the temperature characteristics of thermal exposures so intense that they are capable of burning the skin in a fraction of a second, it was decided to establish by experimentation the reciprocal relationships of time and temperature necessary to destroy cells at lower temperatures and to extrapolate from these data the time curve that should represent the

minimum cell-destroying exposures for higher degrees of temperature.

17.6.1 Method of Controlling Surface Temperature

Direct exposure of the surface of the skin to a rapidly flowing stream of hot liquid was chosen as the method best adapted for the acquisition of these data. With this type of exposure, the surface of the skin could be maintained at the temperature desired without the establishment of an appreciable gradient between it and the source of heat. There was no insulation of the surface by a static layer of gas, liquid, or solid, no heat loss through vaporization of surface moisture, and no diminution of subsurface heat conduction due to vascular occlusion by the application of pressure on the surface. The method was simple to operate and led to remarkably reproducible cutaneous effects.

The applicator by which a running stream of hot water was brought into direct contact with the skin consisted of a metal cup, the brim of which was covered with a pad of closed-cell sponge rubber to insure a watertight contact. By means of an electric pump, water was circulated from a large constant-temperature reservoir through the cup, the open end of which was applied to the skin. The rate of flow was regulated by a screw clamp on the inlet tube and by the height of the outlet tube (see Figure 12).

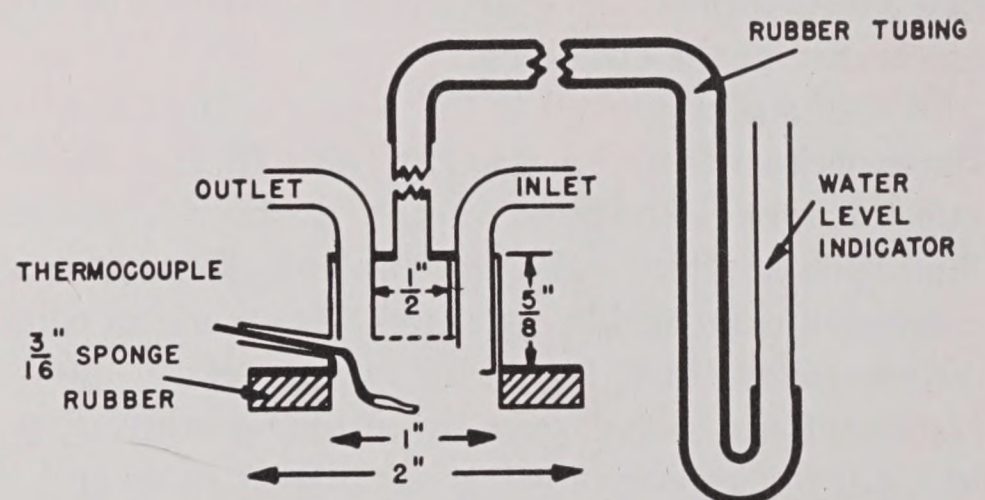


FIGURE 12. Drawing of hot water applicator.

Tangential flow of a liquid produced no vertical component of force and thus no vertical pressure. Vertical water pressure within the cup could be varied between 70 and 86 cm of mercury by suitable adjustments of the aperture of the inlet and the height of the outlet tubes. A copper-constantan thermocouple measured the temperature of the water flowing next to the skin. During any period of exposure the temperature of the water flowing over the skin could be controlled to within 0.1 C.

Two methods were used to equilibrate the apparatus before applying it to the skin. In one, the apparatus was applied to a block of linoleum, adjusted to the desired pressure, and transferred to the skin site to be exposed as soon as the temperature equilibrium was reached. In the other, the applicator was allowed to remain immersed in the hot water reservoir with the pump turned on until thermal equilibrium was established. The cup was then transferred immediately to the skin and adjusted to the desired water pressure.

Provision was made in the construction of this apparatus for studying the relation of the size of the area of exposure to the intensity of the resultant injury. This was accomplished by making the brim of the cup removable so that the area of skin to be exposed could be varied according to the aperture size of the brim selected for use. Thus, in the same region on the same animal and under identical conditions of time, temperature, and pressure, circular targets having a diameter of either 7 or 25 mm could be exposed.

Individual burns in the animal experiments were 25 mm in diameter. This was larger than desirable for human subjects and the diameter of the aperture of the cup was accordingly reduced to 7 mm for the human experiments. Before this was done, however, it was established by animal experimentation that the reduction in the size of the exposure area did not make any appreciable difference in the effect of such exposures on the epidermis.

Water was employed as the source of heat in all of the experiments summarized in Table 10. Because the question was raised as to whether or not a hypotonic fluid such as water might modify the effects of heat, a series of comparable exposures were made in which oil was substituted for water. There was no appreciable difference between the injury-producing potentiality of rapidly flowing streams of water and oil on either animal or human skin so long as the temperature and duration of exposure were the same.

17.6.2 Experiments on Pigs

The primary purpose of this investigation was to obtain information relating to the tolerance of human skin to episodes of hyperthermia of varying duration and of varying degrees of intensity, and the direct approach would have been to make all experiments on human subjects. For various reasons, this was not feasible, and it was decided to acquire the basic data from experiments on pigs. From an ex-

tensive series of observations on pigs, it was thought that a relatively small number of critical exposures of human skin would establish the extent to which the more comprehensive animal data were applicable to man.

Closely clipped young (8 to 12 kg) white pigs were used. It was found that different portions of the body surface of the pig vary slightly in respect to their susceptibility to thermal injury. The largest uniformly reacting area was the lateral body surface beginning in front of the thighs and extending forward over the shoulders. The skin of the neck and for about 10 cm to either side of the spine had a slightly higher thermal tolerance than that of the lateral body surface. The skin covering the thighs, the buttocks, the inguinal folds, and the mid-portion of the chest and abdomen had a slightly lower thermal tolerance.

Results of experiments on pigs: The surface temperature, duration, and results of 179 hot water applications to the lateral body surface of young white pigs are summarized in Table 10.

The surface temperatures at which these exposures were made ranged between 44 and 100 C. The duration of exposures varied between 1 second and 7 hours. The majority of the exposed sites were kept under observation until the reaction had subsided or the lesion had healed. In the case of borderline reactions duplicate exposures were made and excised at the end of 24 or 48 hours for microscopic study.

As indicated in Table 10, a wide variety of reactions were observed. These ranged in severity from evanescent erythema to deep ulcers.

In the beginning certain difficulties were encountered in recognizing differences in the severity of certain lesions. Although there was no difficulty in recognizing the difference between a reaction whose total effect was a mild and transient erythema and one that led to deep coagulative necrosis, it was not always easy to recognize by clinical observations whether a given lesion represented a severe first-degree reaction with incomplete or focal epidermal destruction or a relatively mild second-degree reaction in which the epidermal destruction was complete.

Apart from the microscopic appearance, the most reliable criteria by which to recognize transepidermal necrosis were (1) the ease with which dead but still intact epidermis could be displaced by friction on the second and third days after exposure, and (2) the development of complete encrustation of such a lesion within a week.

TABLE 10. Time-surface temperature thresholds for thermal injury of porcine skin.

Temp C	Time Min Sec	No. of expt	Subthreshold exposures		Threshold and supra-threshold exposures	
			1° reactions		2° and 3° reactions	
			Hyperemia only	Focal epidermal necrosis	Complete epidermal necrosis	
			Mild Severe	Scal- ing Small ulcers	Red burn Pale burn	
44	420	1		+		
45	150	1	+			
	180	1			+	
46	45	1	+			
	60	1		+		
	90	1			+	
46.5	45	1	+			
	60	1			+	
47	35	1	+			
	45	1			+	
	50	1			+	
	60	1			+	
48	10	3	+			
	12	1		+		
	14	2			+	
	14	1			+	
	15	2			+	
	16	1		+		
	18	1			+	
	20	1			+	
49	3		+			
	4	5	+			
	5	2				
	6	5		+		
	6	2			+	
	6	2			+	
	7	2		+		
	7	1			+	
	7	1			+	
	8	4		+		
	8	1			+	
	8	2			⊕	
	9	11			+	
	10	5			+	
50	1	1	+			
	2	1	+			
	4	1		+		
	5	1		+		
	5	3		+		
	5	2			+	
	5	2			+	
	6	2			+	
	6	30			+	
51		45	2	+		
	1	2	+			
	1	30	2	+		
	2	1		+		
	3	2		+		
	3	2		+		
	3	2			+	
	4	2			+	
	5	1			+	
	5	1			+	
	10	2			+	
52		30	1	+		
		45	1		+	
	1	30	1		+	
	2		4		+	
	3		1		+	
53		20	1	+		
		30	1		+	
		45	2		+	
	1		2			+
	1	30	3			+
	2		1			+
54		15	1	+		
		25	1		+	
		35	1			+
55		5	1	+		
		10	1	+		
		15	1	+		
		20	1		+	
		25	1			+
		30	3			+
56		10	1		+	
		15	1			+
		20	1			+
58		5	1	+		
		10	1			+
60		2	1	+		
		2	1		+	
		3	1		+	
		5	1			+
		7	1			+
		7	1			+
		10	2			+
	10		1			+
65		1		+		
		2	1			+
		3	1			+
	10		1			+
70		1	2			+
		2	1			+
	3		2			+
75		1	1			+
		5	1			+
80		1	1			+
		5	1			+
85		1	1			+
		5	1			+
90		1	1			+
		5	1			+
95		1	1			+
		3	1			+
100		1	1			+
		3	1			+

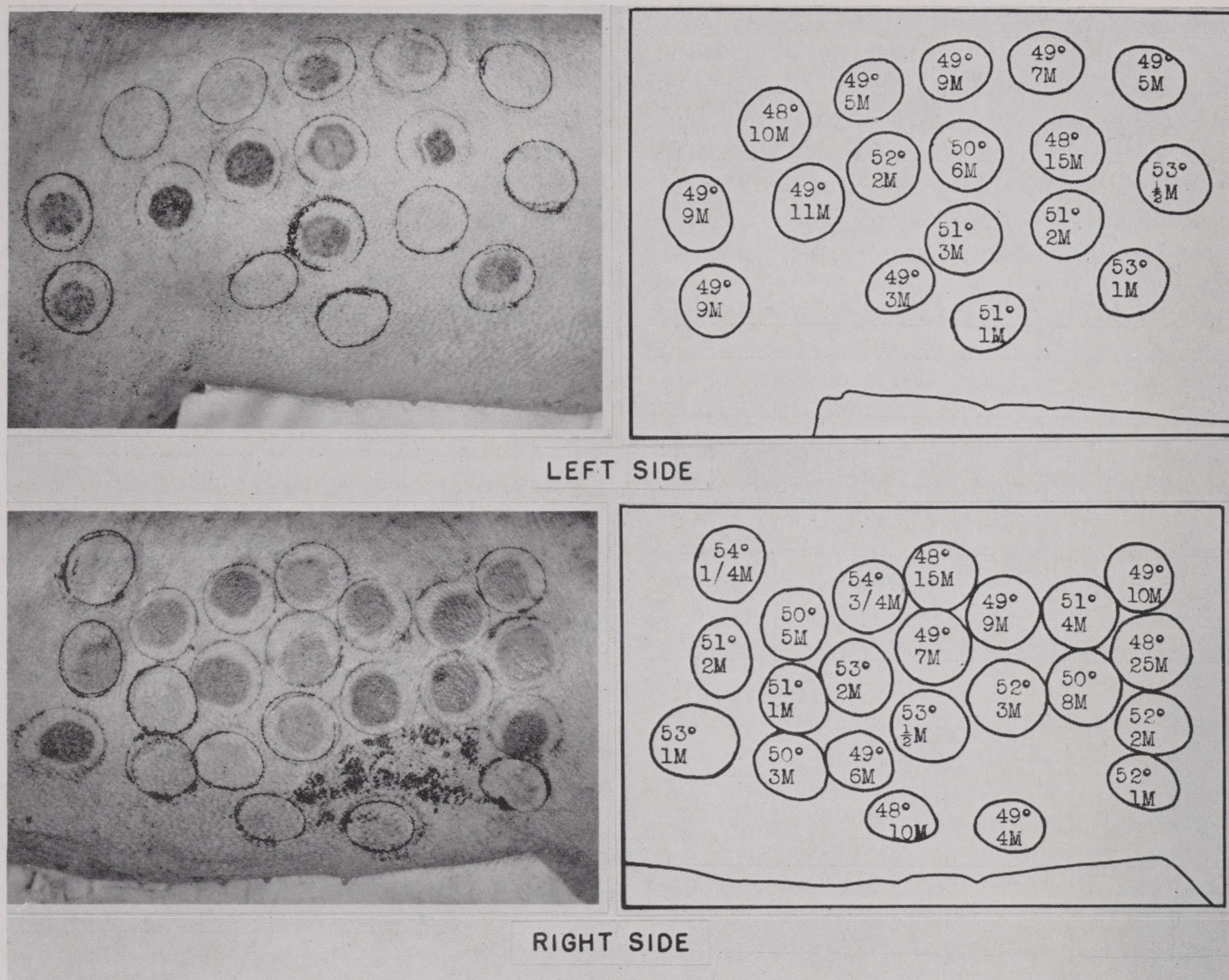


FIGURE 13. Photograph of right and left sides of pig with temperature and duration of each exposure indicated. Lesions on right side were 24 hours old and those on left side 7 days old.

All exposures sufficient to cause vascular reaction but insufficient to destroy the full thickness of the epidermis throughout the entire target area were designated as subthreshold. The entire range of cutaneous responses to subthreshold exposures were characterized as first-degree reactions. The shortest time at any given temperature that was capable of causing transepidermal necrosis constituted a threshold exposure. The effect of a threshold exposure on the skin was characterized as a second-degree reaction. All exposures which were of longer duration or higher temperature than was necessary to cause complete epidermal destruction were designated as suprathreshold and their effects as third-degree reactions.

The macroscopic appearance of different degrees of cutaneous reaction to hyperthermia may be seen in the photographs of the right and left sides of pig 924

shown in Figure 13. At the time the photographs were made, the lesions on the right side were 24 hours old and those on the left were 7 days old. It is apparent from these photographs that the duration of exposure at any given temperature was remarkably critical in relation to the kind of reaction evoked. It is equally apparent that the time required to produce a given degree of reaction varied inversely with the temperature.

17.6.3 Experiments on Human Subjects

In order to determine the extent to which the reciprocal relationships of time and temperature in the production of cutaneous burns in pigs were applicable to human skin, a series of exposures similar to those described on pigs were made on human subjects. Some were made to the skin of the anterior thoracic region and others on the ventral aspect of the fore-

arm. The applications were made with the apparatus shown in Figure 12.

As in the case of the pig experiments, three degrees of skin reaction were observed. Reactions characterized as first-degree were those that fell short of complete destruction of the epidermis. At one extreme a first-degree reaction consisted of a faint and transient erythema. At the other, extreme erythema was severe and prolonged and miliary vesicles formed but failed to coalesce. Lesions in which there was complete destruction of the epidermis over the entire target area were designated second- or third-degree reactions, depending on the extent to which the dermis was involved. As in the case of the pig, a threshold exposure represented the shortest time at any given temperature that caused complete destruction of the epidermis.

That a given exposure of human skin had resulted in transepidermal necrosis was usually but not always recognized by complete vesication of the target area. Although vesication resulting from heat indicates that the full thickness of the epidermis has been destroyed, absence of vesication does not necessarily indicate that the epidermis has escaped complete destruction. Transepidermal necrosis without vesication was observed after certain suprathreshold exposures. The explanation of this phenomenon will be discussed in Section 17.8.8.

The results of the human experiments have been summarized in Table 11.

17.6.4 Relative Vulnerability of Porcine and Human Skin to Thermal Injury

To facilitate comparison of the data included in Tables 10 and 11, certain of the more critical observations have been depicted graphically in Figure 14. The solid line was established by points representing the time and temperature of exposures that caused minimal second-degree reactions of porcine skin. The points by which this line was established are represented by crosses. Each cross represents the shortest time at the temperature indicated that resulted in transepidermal necrosis of the entire target area. The more that the time of any given exposure placed it to the right or that the temperature of any given exposure placed it above the solid line, the greater the depth to which the skin was destroyed. All exposures that were situated a significant distance above and to the right of the solid line were suprathreshold and all those situated a significant distance below and to the

TABLE 11. Time-surface temperature thresholds for thermal injury of human skin.

No.	Temp at sur- face C	Duration of exposure Hr Min Sec			Sub- threshold exposures 1° reac- tions	Threshold and supra- threshold exposures 2° and 3° reactions	Sub- ject	Date
					Hyperemia without loss of epidermis	Complete epidermal necrosis		
1	44	5	..	..	+	..	BF	2/6
2*	..	5	..	..	+	..	BF	2/23
3	..	6	..	..	..	+	BF	2/6
4*	..	6	..	..	..	+	BF	2/23
5*	45	2	..	..	+	+	KL	2/16
6*	..	3	..	..	..	..	KL	2/3
7	..	3	..	..	..	+	HA	2/4
8*	47	..	18	..	..	+	RK†	2/13
9*	..	..	20	..	+	+	KL	2/25
10*	..	..	20	..	+	..	AM	2/26
11*	..	..	20	..	+	..	PG	2/26
12	..	..	25	..	..	+	RK†	1/8
13*	..	..	40	..	..	+	AM	2/26
14	..	..	40	..	..	+	PG	2/26
15	..	..	45	..	..	+	RK†	1/8
16	48	..	15	..	+	..	PG	7/19
17	..	..	15	..	..	+	AR	7/19
18	..	..	18	..	..	+	AM	6/26
19*	49	..	8	..	+	..	AM	2/16
20	..	..	8	..	+	..	AM	6/26
21	..	..	9	30	..	+	AM	6/26
22*	..	..	10	..	..	+	AM	6/26
23	..	..	11	..	..	+	AM	6/26
24	..	..	15	..	..	+	AM	6/26
25	51	..	2	..	+	..	AM	6/26
26	..	..	4	..	..	+	AM	6/26
27	..	..	6	..	..	+	AM	6/26
28	53	..	..	30	+	..	AM	6/26
29	..	..	1	30	..	+	AM	6/26
30	55	..	..	20	+	..	PG	7/19
31	..	..	..	30	..	+	AR	7/19
32*	60	..	..	3	+	..	FH	2/1
33*	..	..	..	5	..	+	FH	2/1

* Oil used instead of water as source of heat.

† Subject RK was atypical in that his threshold for thermal injury was significantly lower than that of other experimental subjects.

left of the solid line were subthreshold. The range of variation is shown in Table 10.

The extent to which the results of human exposure corresponded with those of the more comprehensive animal experiments is indicated by the open and solid circles in Figure 14. The open circles represent the maximum exposure at the temperature indicated that failed to destroy human epidermis and the closed circles represent the minimum time at the temperature indicated that resulted in complete destruction of human epidermis.

The broken line in Figure 14 represents the approximate threshold at which the first morphological

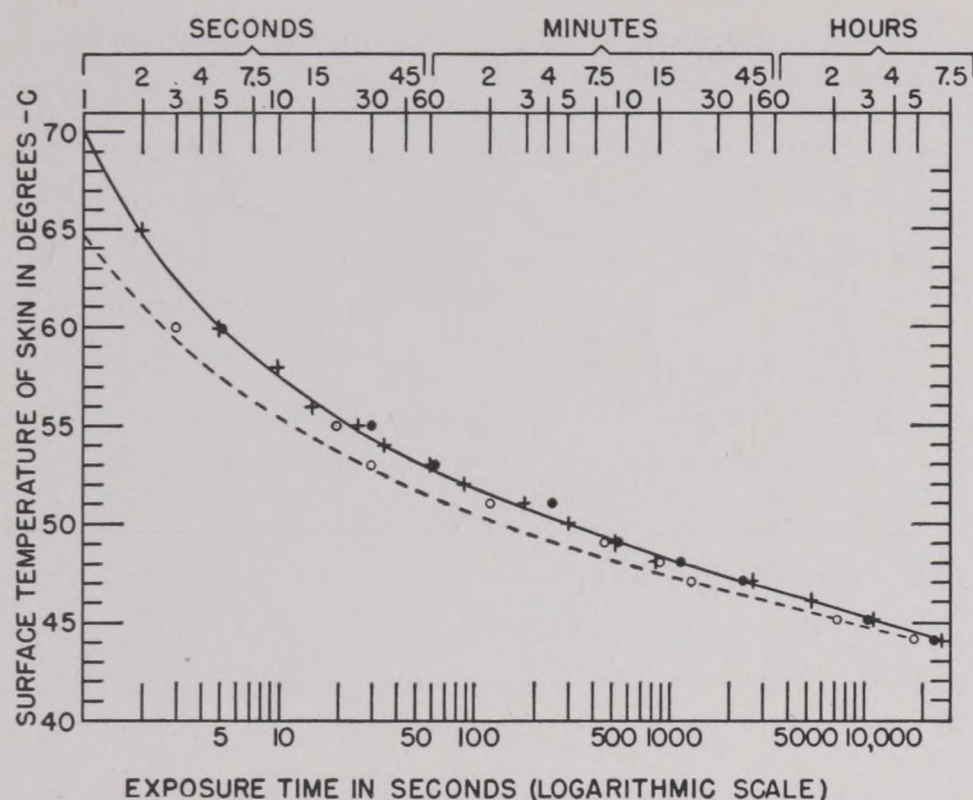


FIGURE 14. Graph showing thresholds for porcine skin at which microscopic evidence of epidermal injury is first apparent (broken line) and at which transepidermal necrosis is complete (solid line). Crosses indicate critical individual experiments and show shortest time at temperature indicated at which transepidermal necrosis of entire target area occurred. Open and solid circles show effects of heat on human skin. Open circles represent longest exposure at temperature indicated that failed to destroy epidermis. Solid circles represent shortest exposure at temperature indicated that resulted in transepidermal necrosis.

evidence of thermal damage to porcine epidermis was recognized. Exposures situated below the broken line caused no appreciable change in the microscopic appearance of the epidermis. Exposures lying between the broken and solid lines resulted in varying degrees of epidermal damage short of transepidermal necrosis. Since the reactions of human skin to control episodes of hyperthermia were not examined microscopically, no inferences can be drawn as to the reciprocal relations of time and temperature at which microscopic evidence of injury to human epidermis was first recognizable.

17.6.5 Mathematical Predictability of Epidermal Destruction by Exposure to Heat^d

From a kinetic standpoint, the reciprocal relationships of time and temperature in the production of transepidermal necrosis follow the general pattern of rate processes. If the reaction leading to thermal death of epithelium conforms to that of most physical and chemical rate processes,²⁰ it should be quantitatively predictable by the following equation:

$$\frac{d\Omega}{dt} = Ae^{-\Delta E/R(T_t + 273)} \quad (7)$$

where $d\Omega/dt$ is the rate at which an arbitrary function

of epidermal injury Ω as determined by histological examination is produced. T_t is the temperature in C at the time, t , at the basal epidermal layer; R is the gas constant and is equal to 2 calories per C per mole; and both A and ΔE are constants evaluated from the experimental data.

Equation (7) can also be expressed as an integral equation, namely

$$\Omega = A \int_0^t e^{-\Delta E/R(T_t + 273)} dt \quad (8)$$

where if T_t , the dependence of the basal epidermal temperature on time, is known the integral can be evaluated.

In all cases where the temperature of the basal layer of epidermis can be considered as independent of time of heat exposure, equation (8) can be integrated to equation (9).

$$\Omega = Ae^{-\Delta E/R(T + 273)t} \quad (9)$$

An examination of the transepidermal threshold data depicted in Figure 14 and the epidermal time-temperature data given in Table 7 (Section 17.3.2) and illustrated in Figure 15 shows that equation (9) is applicable in all heat exposures where the skin surface temperature is less than 50 C; furthermore the skin surface temperature can be substituted for the steady-state basal epidermal temperature since the differences (<0.3 C) between these two values are negligible in this temperature range.

Thus, by using equation (9) in this temperature range, it is possible to evaluate numerically A and ΔE by standard graphical procedures from the data for the threshold of complete transepidermal necrosis; and the following equations are obtained.

$$\Delta E = 150,000 \text{ cal/mole} \quad (10)$$

and

$$A = 3.1 \times 10^{98} \text{ sec}^{-1} \quad (11)$$

This value of A depends upon the arbitrary choice of the value of unity for Ω . Thus, when the threshold of complete epidermal necrosis is reached,

$$\Omega \equiv 1. \quad (12)$$

By again making use of equation (9) a similar analysis can be made of the time-temperature relationship depicted by the broken line of Figure 14. Since these data are not so complete as those used above, it is best to use the same numerical values given by equations (10) and (11) for ΔE and A , and solve for the numerical value of Ω .

These data are found to be best represented by

$$\Omega \equiv 0.53 \quad (13)$$

^d By F. C. Henriques, Jr.

when the upper limit of exposure which can be tolerated without the occurrence of transepidermal necrosis is reached.

Although the values given by equations (10) to (13) for A , ΔE , and Ω were obtained through the use of equation (9), which requires that the epidermal temperature can be considered constant during the entire heat exposure, these numerical values should permit the computation of the two thresholds of transepidermal injury under all conditions by means of equation (8), so long as T_t is known.

Under the experimental conditions that the data depicted in Figure 14 were obtained, namely, constant skin surface temperature during the entire heat exposure, it is possible to ascertain the time dependence of basal epidermal temperature by means of equation (6c). Referring to equation (6c), it is found that the evaluation of T_t depends upon two parameters, T_o and γ . An examination of the equation shows that T_t is very insensitive to variations in T_o , the original epidermal temperature; 35 C is taken as the original skin surface temperature (see Table 6 of Section 17.3.2).

In view of the uncertainties which enter into this direct experimental evaluation of γ by means of equation (6b), it is best to evaluate it empirically by obtaining the best fit to the complete transepidermal necrosis data.

It is then found that

$$\gamma = 0.15 \quad (14)$$

if t , the time during the heat exposure, is expressed in seconds. This numerical value checks well with that obtained by direct substitution of the experimental values for the thermal conductivity, heat capacity, density, and thickness of epidermis (see Section 17.3.2) into equation (6b).

A consideration of equations (6a), (6c), and (8) together with the requisite numerical values given by equations (10), (11), and (14) shows that the experimental data given in Table 10 and depicted in Figure 14 are completely described by the following equation:

$$\Omega = 3.1 \times 10^{98} \int_0^t e^{-75,000/T_t + 273} dt \quad (15)$$

where

$$T_t = T_s - (T_s - 35) \left[\frac{2}{\sqrt{\pi}} \int_0^{0.15/\sqrt{t}} e^{-y^2} dy \right] \quad (15a)$$

where Ω is the degree of injury to be expected, T_s is

the surface temperature of the skin during heat exposure, T_t is the temperature of the basal epidermal layer after the time t in seconds has elapsed, and the numerical values of the integral of equation (15a) as a function of $0.15/\sqrt{t}$ are tabulated.³⁹

For $\Omega > 0.5$ and $T_s < 50$ C the time dependence of T_t can be ignored and T_t put equal to T_s ; equation (15) can then be integrated and takes the form of equation (9), which greatly facilitates the computation of Ω . For all $T_s > 50$ C and $\Omega \leq 1$, the time dependence of T_t cannot be neglected, and the evaluation of Ω by means of equation (15) requires one of the standard methods of numerical integration.¹⁷

This numerical determination of Ω from the two experimental parameters, t and T_s , permits the prediction of the degree of epidermal injury, since an $\Omega \leq 0.53$ results in a time-temperature relationship that can be tolerated without the occurrence of transepidermal necrosis, and $\Omega \geq 1.0$ results in a time-temperature relationship which produces complete epidermal necrosis.

The success of equations (15) and (15a) in predicting these time-temperature relationships is shown in Table 12.

It can be seen that the agreement of the experimental data of Section 17.6.3 with this equation is, in general, excellent, and, thus, that the tacit assumption throughout this section of the applicability of equation (7) is justified. In the four cases where there is appreciable variance between experiment and prediction, either the experimental data are insufficient or the duration of heat exposure was too short to preclude considerable experimental error. Thus, equations (15) and (15a) probably give a more accurate prediction of epidermal injury thresholds than the dotted and solid lines of Figure 14.

The numerical computations resulting from equation (9) are also included for comparative purposes. For the reasons stated above there is no appreciable difference, under these specific experimental conditions, between this equation and equation (15) for all surface temperatures below 50 C. Equation (9) corresponds to an experimental condition in which the basal epidermal layer is immediately brought to and maintained at a constant temperature. If this were feasible at 70 C, complete epidermal necrosis would result in 3 ten-thousandths of a second. The 2,000-fold difference between this value and 0.5 second predicted by equations (15) and (15a) indicate the extreme importance of the heat capacity of the skin during the early period of heat exposure.

TABLE 12. A comparison of the experimental time-temperature relationship for transepidermal injury as depicted by Figure 14 with those obtained from equations (9) and (15).

Minimum time in seconds for complete transepidermal necrosis $\Omega \equiv 1$			Maximum time in seconds for subthreshold transepidermal necrosis $\Omega \equiv 0.53$			
Equation (9)*	Equation (15)	Experimental solid line Figure 14	Surface temp C	Experimental dotted line Figure 14	Equation (15)	Equation (9)*
23,000	23,000	25,000	44	18,000†	12,000	12,000
11,000	11,000	11,000	45	7,200†	5,900	5,800
5,100	5,200	5,000	46	3,000	2,800	2,700
2,400	2,500	2,400	47	1,300	1,380	1,300
1,100	1,200	1,100	48	560	650	600
580	630	570	49	260	340	310
270	325	300	50	130	165	140
130	165	160	51	75	90	68
65	91	90	52	44	52	35
16	31	35	54	18	19	8
4.4	13	16	56	8.3	8.1	2.3
0.25	3.0	5	60	2.6	2.3	0.13
0.009	1.0	2†	65	1.0	0.7	0.005
0.0003	0.5	1†	70		0.4	0.0002

* Above 50 C equation (9) has no experimental significance.
† Experimental value uncertain.

These tabulated values, resulting from the solution of equation (15), are of course only valid under specific experimental conditions, namely, only when the skin surface temperature is immediately brought to and maintained at a constant value during the entire heat exposure. However, equation (15) should accurately predict the epidermal injury to be expected from all conceivable kinds of heat exposures, so long as the temperature dependence of the skin surface temperature as a function of time is known, since the time-temperature relationship at the basal epidermal layer can be predicted quite accurately by making use of the “infinite body” heat theory¹¹ implicit in equation (15a). (See Section 17.3.1.)

17.6.6 Vulnerability of Ischemic Skin to Thermal Injury

One of the reasons that a running stream of hot water was employed as the source of heat in these experiments was that by this technique there would be no mechanical interference with the circulation of blood through the dermal capillaries. The exposures were made at atmospheric pressure. It was felt that circulation of relatively cool blood through the dermal capillaries would probably tend to protect the skin against burning and that any method employed for the production of uniform burns should be one which did not cause mechanical interference with capillary circulation.

The following experiments were undertaken for the

purpose of determining the extent to which local impairment in blood flow may increase the vulnerability of the epidermis to thermal injury.

A control series of burns were made on each of three pigs by exposing various skin sites to hot water at atmospheric pressure. The predetermined time and temperature of each exposure were such that severe first-degree or mild second-degree reactions could be anticipated. See Table 13.

TABLE 13. Effects of thermal exposures with and without pressure ischemia.

Animal number	Expt temp C	Exposure duration (min)	Excess pressure on skin (mm Hg)	No. of exp made	Number of lesions Without transepi-dermal necrosis	With transepi-dermal necrosis
887	49	7	0	5	5	0
	49	9	0	5	0	5
	49	7	80	5	5	0
899	49	7	0	4	4	0
	49	8	0	4	2	2
	49	9	0	4	0	4
	49	7	80	4	4	0
	49	8	80	4	3	1
	49	8	80	4	3	1
901	51	2	0	3	3	0
	51	3	0	3	2	1
	51	4	0	3	0	3
	51	2	80	3	3	0
	51	3	80	3	1	2

It was found that all 7-minute exposures at 49 C and all 2-minute exposures at 51 C made at atmospheric pressure were subthreshold in the sense that

they failed to cause complete transepidermal necrosis. That they were close to threshold was indicated by the fact that all 9-minute exposures at 49 C and all 4-minute exposures at 51 C did cause transepidermal necrosis.

After it was established that the position of the threshold for transepidermal necrosis in these animals was between 7 and 9 minutes at 49 C and between 2 and 4 minutes at 51 C for exposures made at atmospheric pressure, a second series of exposures were now made during which the water pressure was increased by an amount corresponding to 80 mm of mercury. With this amount of pressure on the surface of the skin during the time that it was exposed to heat, there was no instance in which the reaction to a 7-minute exposure at 49 C or to a 2-minute exposure at 51 C was increased in severity.

It is apparent from the data summarized in Table 13 that the application of pressure sufficient to collapse superficial dermal capillaries during a period of exposure does not cause appreciable augmentation in the vulnerability of epidermis to thermal injury.

In view of the extreme thinness of the epidermis, these results were to be expected, since, for reasons given in Section 17.3.2, the epidermal temperature is primarily determined by the skin surface temperature. Thus the dermal temperature gradients, which may be appreciably altered in ischemic as compared with normal skin during thermal exposure, would have little effect on the time-temperature relationship that exists at the epidermal-dermal interface.

17.6.7 Latent Thermal Injury and Cumulative Effects of Repeated Subthreshold Exposures

If the data summarized graphically in Figure 14 are recalled it is apparent that recognizable damage to the epidermis occurred only during the terminal phase of the subthreshold exposures represented in these experiments. Not until the duration of any given episode of hyperthermia was such as to bring it to the level indicated by the interrupted line in Figure 14 was there recognizable evidence of epidermal injury. This phenomenon is even more readily apparent in the photographs shown in Figure 13. In these, it may be seen that the 7-minute exposure at 49 C on the left side of the animal shows only a trace of residual erythema, whereas both of the sites of 9-minute exposures at that temperature show transepidermal necrosis.

Does this indicate that no epidermal injury had

been sustained during the first 7 minutes, or does it mean that injury was present but morphologically latent?

In order to gain more information concerning this point, the experiments summarized in Table 14 were undertaken. Thermal exposures were made with a running stream of hot water at 49 C and at atmospheric pressure. Three young pigs were used.

The first series of exposures (1 to 18) were for control purposes and served to establish the reproducibility of reactions to single exposures at this temperature. It may be seen that there was not a single instance in which an exposure for less than 7 minutes caused recognizable necrosis of the epidermis and that in every instance in which exposures as long as 9 minutes were given, there was complete necrosis of the epidermis. Skin sites receiving 7-minute exposures recovered without loss of epidermis, whereas skin sites receiving 9-minute exposures underwent complete ulceration.

The control exposures were followed by a series (19 to 39) in which repeated exposures, individually incapable of causing recognizable epidermal injury, were applied to the same area. It was found, for instance, that, although a single 3-minute exposure at 49 C caused no recognizable change in the epithelial cells, three such exposures separated by recovery periods as long as 24 minutes had the same total destructive capacity as a single continuous 9-minute exposure.

It was clear that a certain amount of epidermal injury was sustained during the first 3 minutes and that at least 24 minutes were required before there was an appreciable recovery from this injury. That complete recovery occurred after between 2 and 4 hours was indicated by experiments 30 and 31.

Experiments 34 to 39 showed what might have been expected, namely, that recovery from the latent injury of a 2-minute exposure was more rapid and that from a 5-minute exposure less rapid than was the case after a 3-minute exposure.

Further discussion of the implications of these experimental results will be found in Section 17.8 of this chapter.

17.6.8

Summary

The reciprocal relationships of time and temperature in the causation of transepidermal necrosis are similar for similar skin areas of man and pig.

Thermal injury or burning of the skin was observed to occur at surface temperatures as low as

TABLE 14. The cumulative effects of repeated subthreshold thermal exposures on the skin of the pig. All exposures were made to water at 49 C.

Duration of each exposure (min)	No. of exposures at same site	Interval between exposures	Effect of exposure on skin				Expt No.
			No evidence of epidermal injury		Epidermal necrosis		
			Mild vasc. reaction	Severe vasc. reaction	Focal	Complete and irreversible	
3	1	...	+	...	..	..	1
..	1	...	+	...	..	..	2
..	1	...	+	..	..	..	3
4	1	...	+	..	..	..	4
5	1	...	+	..	..	..	5
6	1	...	..	+	..	..	6
..	1	...	..	+	..	..	7
..	1	...	..	+	..	..	8
7	1	...	..	+	..	..	9
..	1	...	..	..	+	..	10
8	1	...	..	..	+	..	11
..	1	...	..	..	+	..	12
..	1	...	..	..	..	+	13
9	1	...	..	..	..	+	14
..	1	...	..	..	..	+	15
..	1	...	..	..	..	+	16
..	1	...	..	..	..	+	17
..	1	...	..	..	..	+	18
3	3	3 min	..	..	..	+	19
..	3	3 min	..	..	..	+	20
..	3	3 min	..	..	..	+	21
3	3	6 min	..	..	..	+	22
3	3	12 min	..	..	..	+	23
3	3	24 min	..	..	..	+	24
3	3	48 min	..	..	..	+	25
..	3	48 min	..	..	+	..	26
3	3	72 min	..	..	+	..	27
..	3	72 min	..	..	+	..	28
3	3	96 min	..	..	+	..	29
3	3	120 min	..	+	..	..	30
3	3	240 min	+	..	..	..	31
3	3	24 hr	+	..	..	..	32
3	3	48 hr	+	..	..	..	33
2	5	2 min	..	..	..	+	34
..	5	30 min	+	..	..	..	35
..	5	60 min	+	..	..	..	36
3	2	12 min	..	+	..	..	37
5	2	60 min	..	..	..	+	38
..	2	240 min	..	..	+	..	39

44 C and it can be inferred from the shape of the time-temperature curve that the threshold at which hyperthermal cellular injury is first sustained is not far above the level that is normal for the blood.

The rate at which injury occurs increases rapidly if the temperature is raised. The progress of injury at any given temperature is determined by the duration of the hyperthermic episode. Thus, the amount of time required to convert a reversible into an irreversible cellular injury is different for each degree of temperature and in the case of the epidermis can be computed if the surface temperature as a function of time is known.

The existence of latent or morphologically unrecognizable cellular injury after certain apparently harmless thermal exposures and the fact that the time required for recovery from such latent injuries becomes longer the nearer they approach the threshold of microscopic visibility were demonstrated experimentally.

17.7 PATHOLOGY OF CUTANEOUS BURNS AND THEIR PATHOGENESIS^{3c}

In the foregoing section, measurements of the reciprocal relationships of time and surface temperature with respect to the capacity of thermal exposures

to destroy the epidermis were reported. The following studies concern the pathological characteristics of cutaneous injuries caused by thermal exposures at different temperatures and for different durations, and a comparison of the pathogenesis of cutaneous burns in man and pig.

17.7.1 Experimental Procedure

Much of the material used in this investigation was derived from the experiments described in Section 17.6 of this chapter. It was added to from several sources (see Table 15). Since most of the lesions produced in the experiments reported in Section 17.6 were not excised until they had been under clinical observation for days or weeks, many duplicate exposures were made and excised in order to observe in the various types of lesions the sequence of microscopic changes that took place between injury and repair. To acquire this material, approximately 60 additional hot water exposures of pigs were made and examined microscopically after recovery periods ranging between a few seconds and several weeks. Additional material comprised a series of burns of porcine skin made by exposure to hot air at temperatures varying between 80 and 900 C. There were two series of human burns, one comprising 33 experimentally produced lesions which were studied clinically but were not excised for microscopic examination, and the other comprising a collection of skin specimens obtained after death from victims of conflagrations.

Sections of tissue for microscopic examination were cut from specimens that had been fixed in Zenker-formol or 10 per cent formaldehyde. Phloxine-methylene blue stains were made routinely and were augmented by sections stained with hemotoxylin and eosin or by Pollak's modification of Masson's trichrome method. Many sections were stained by the Feulgen technique.

TABLE 15. Sources and kinds of material used for study of the pathogenesis of cutaneous burns.

Sub- ject	Source of heat	Range of temp C	Range of exposure	Range of recovery period
Pig	Water	44-100	0.5 sec -7.5 hr	1 min-4 weeks
Pig	Air	80-900	0.5 min-45 min	1 min-3 days
Man*	Water or oil	44-60	3 sec-6 hr	1 min-4 weeks
Man	Air	?	?	Less than 1 hr

* Lesions not excised for microscopic study.

17.7.2 General Consideration of Quantitative and Qualitative Effects of Heat on Skin

A cutaneous injury caused by hyperthermia may be characterized quantitatively according to the depth to which the tissue has been destroyed, or qualitatively according to the nature of the changes that have occurred. The characterization in Section 17.6 of hyperthermic episodes as subthreshold, threshold, and suprathreshold referred to their quantitative capacities for injury production, the determining factor being the capacity of the exposure to cause complete destruction of the epidermis.

Thus, any exposure that failed to cause complete destruction of the epidermis was designated as subthreshold and any reaction short of transepidermal necrosis was one of the first-degree. A second-degree reaction was one caused by the shortest exposure at any given temperature, or by the lowest temperature at any given time, that resulted in full-thickness destruction of the epidermis. Although it was not possible to destroy the entire thickness of the epidermis without some damage to the underlying connective tissue, dermal necrosis was a relatively insignificant feature of a truly threshold exposure. A third-degree reaction was one caused by an exposure that was suprathreshold in respect to time or temperature and was accordingly one in which a significant degree of dermal necrosis usually accompanied the destruction of the epidermis.

SLOPE OF TRANSCUTANEOUS TEMPERATURE GRADIENT IN RELATION TO DEPTH AND QUALITY OF BURN

If account is taken of the potential variations in the intensity and duration of the different thermal exposures that are capable of producing burns of similar depth, it becomes apparent why thermal lesions of approximately the same depth may be qualitatively dissimilar.

This fact is more readily appreciated by reference to Figures 15 and 16. The critical temperature, so far as the ultimate fate of the epidermis is concerned, is that attained at the interface between epidermis and dermis rather than that of the surface. In Figure 15 are shown the estimated changes^e in temperature that would occur at the basal cell level during the course of thermal exposures at three different surface temperatures if each were terminated at a time calculated to be just adequate to destroy the epidermis. In Figure 16 are shown the temperatures that would

^e Calculations based on data presented in Section 17.3.

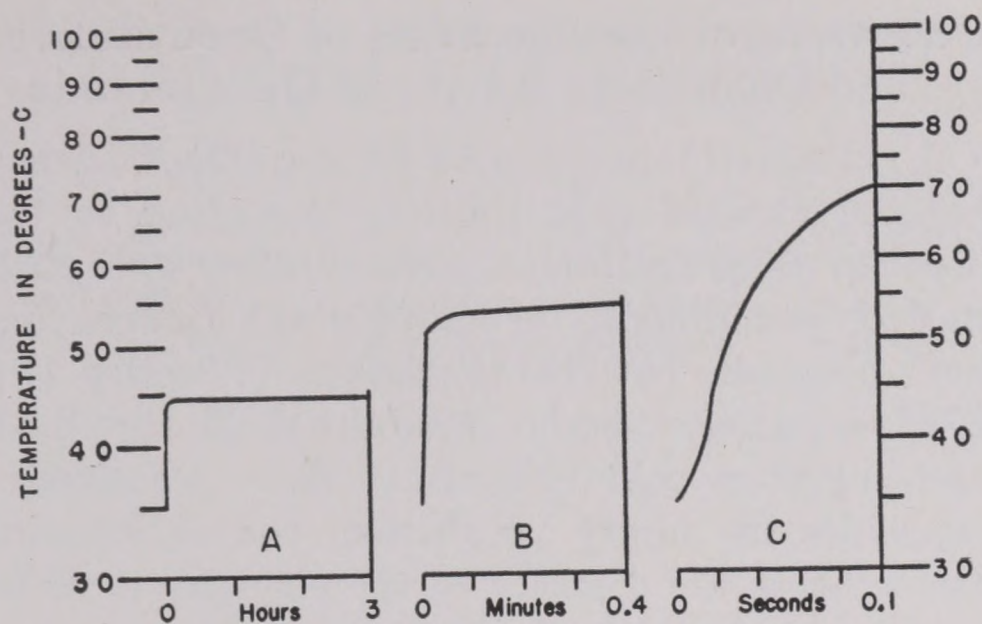


FIGURE 15. Curves depicting changes in temperature at interface between dermis and epidermis during surface exposures of 45 (A), 55 (B), and 100 (C) C. Each of these was threshold exposure in that 3 hours, 0.4 minute, and 0.1 second, respectively, are estimated to be shortest time at indicated temperature that would cause transepidermal necrosis. (Estimates derived from measurements reported in Section 17.3.2.)

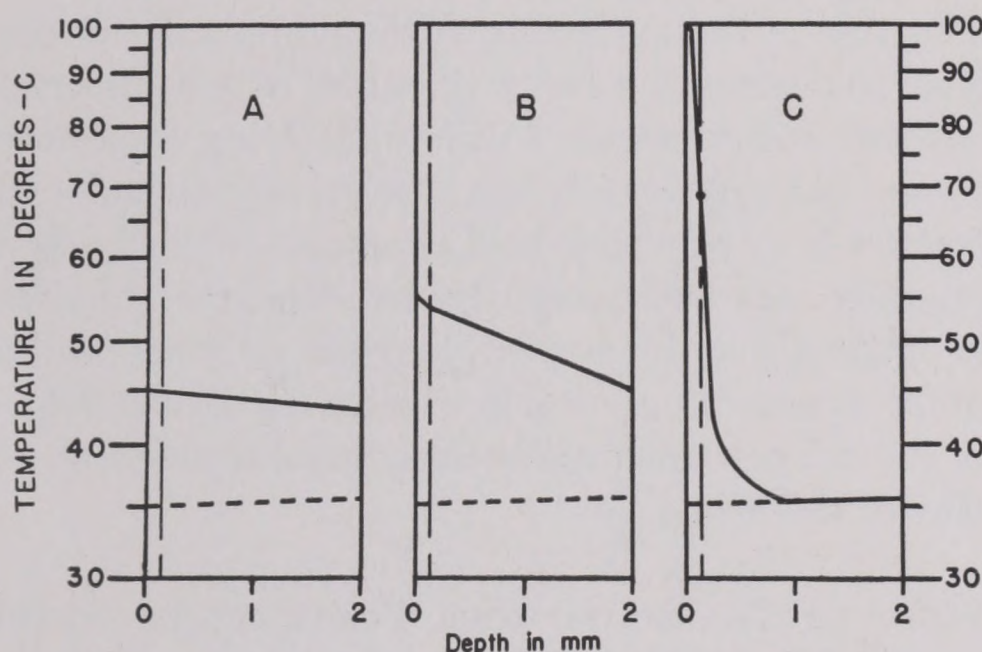


FIGURE 16. Solid line traversing each chart from left to right depicts temperature gradient through first 2 mm of skin at conclusion of exposures estimated to be just sufficient to cause transepidermal necrosis. Interrupted line traversing each chart from left to right depicts original pre-exposure temperature gradient through skin to depth of 2 mm. The 0 vertical line in each represents surface of skin. Interrupted vertical line at depth of approximately 0.1 mm indicates depth of dermal-epidermal interface. In A, surface temperature of 45 C had been maintained for 3 hours. In B, surface temperature of 55 C had been maintained for 0.4 minute. In C, surface temperature had been maintained at 100 C for 0.1 second. (Estimates derived from measurements reported in Section 17.3.2.)

prevail at different depths below the surface of each at the moment that the duration of the exposure was just sufficient to cause irreversible injury of the entire thickness of the epidermis.

In each instance, the effects would be quantitatively similar, in that irreversible cellular injury

would extend to, but not far beyond, the basal cell layer. That qualitative differences in the resulting reactions might exist despite their quantitative similarity can be inferred from the fact that in the exposure shown in Figure 15A the epidermis was destroyed by a 3-hour episode of hyperthermia the intensity of which at no time rose above 44.8 C at the basal cell level. Approximately the same amount of irreversible change would be sustained as the result of the exposure depicted in 15 C. In the latter instance, the epidermis was destroyed in approximately 0.1 second by an episode of hyperthermia in which the temperature at the basal cell level rose sharply and briefly to 70 C. The exposure depicted in 15B falls about midway between these extremes. Although the total amount of irreversible injury is about the same in each, it is not surprising that the three lesions produced in this manner differed qualitatively.

Since certain qualitative characteristics of thermal reactions are dependent on the degree to which the temperature of the tissue has been raised, it follows that the longer any given episode of tissue hyperthermia is maintained, the greater the likelihood that the qualitative attributes of the reaction will reflect the intensity of the exposure.

Such was found to be true: The more severe the exposure, the greater were the qualitative differences between the reactions produced at high and low surface temperatures.

An additional reason for the occurrence of qualitative differences in quantitatively similar reactions to thermal exposures of different intensity is shown in Figure 16. There are depicted the calculated transcutaneous thermal gradients to a depth of 2 mm that would exist at the moment of completion of the same three episodes of hyperthermia illustrated in Figure 15. In each instance, irreversible thermal injury would extend to, but not appreciably beyond, the basal cell layer. In the exposure depicted in A (Figure 16), the temperature of the dermis to a depth of about 2 mm was elevated above normal for at least 2 hours. In the exposure depicted in C, the transcutaneous thermal gradient was so steep that the resulting temperature changes in the dermis were exceedingly brief and superficial. It is apparent why the epithelial cells would be destroyed in C with relatively little disturbance of the dermis, whereas, in A, the same or even a lesser degree of epidermal injury might be accompanied by a severe and persistent vascular disturbance.

17.7.3 First-Degree Reactions

HYPEREMIA, EDEMA, AND CYANOSIS

Sufficient dilatation of the superficial capillaries to cause erythema characteristically accompanied and frequently preceded damage to the epithelium. One exception to this generalization represented in the material upon which this investigation was made was provided by the effects of heat on the skin of animals suffering from circulatory failure. In the presence of circulatory failure, there was frequently such a profound depression of vasomotor irritability that injurious episodes of either high- or low-intensity hyperthermia failed to elicit vascular reactions even though extensive epidermal injury was sustained. Other circumstances in which thermal damage of the epidermis was sustained with little or no accompanying vascular reaction included exposures of sufficient intensity to burn the stratum corneum, but so brief as to cause little or no rise in dermal temperature.

Attention has already been called to the fact that the duration of an episode of low-intensity hyperthermia must be greatly prolonged if it is to produce an injury quantitatively comparable to one resulting from a high-intensity exposure. Since the dermal blood vessels are far more responsive to temperature changes than are the epithelial cells, it can be understood why severe and persistent vascular reactions were often elicited by protracted episodes of low-intensity hyperthermia that failed to harm the epidermis (see Figure 13).

There was considerably greater individual variation among human subjects than there was among pigs in respect to the vascular reactions to cutaneous hyperthermia. The variability of dermal vascular reactions in human subjects was so great and the number of reactions studied in this investigation was so few that little could be inferred as to the extent to which the animal data apply to human skin. The impression was gained that the thermal stimulus necessary to cause visible erythema in most human subjects was substantially lower than that required to elicit erythema in pigs. In man the change in skin color was usually more intense and of longer duration than that in the pig after an identical exposure.

That an active circulation of blood was maintained through the dilated capillaries of an evanescently erythematous skin was indicated in part by the pink or red color of the surface and in part by the fact that the surface temperature during such a reaction was characteristically between 0.5 and 1.5 degrees higher than that of the adjacent skin.

An evanescent erythematous reaction to heat could not as a rule be recognized in sections prepared for microscopic examination. Vessels, the seat of physiological dilatation, usually contracted during or immediately after excision, and it was difficult or impossible to distinguish a sample of physiologically hyperemic skin from one that was normal or ischemic.

If cutaneous hyperthermia was prolonged to between 40 and 60 per cent of the minimum time required for the production of transepidermal necrosis in either man or pig, it characteristically resulted in a more severe and pathological vascular disturbance which led to edema and cyanosis and which persisted for days rather than minutes or hours. That the flow of blood through the dilated capillaries was slowed was indicated by the blue or purple color of the surface in contrast to the pink or red color caused by the more evanescent active hyperemia. The surface temperature of such a lesion during the first few hours was frequently found to be from 0.5 to 2 degrees below that of the adjacent normal skin. That the reaction was pathological rather than physiological was also indicated by the fact that in both man and pig it was almost invariably accompanied by cutaneous edema. Within the first hour after the onset of a vascular injury of this grade, the water content of the dermis was observed to increase by as much as 100 per cent.

Microscopic examination of reactions of this type at varying periods after exposure in the pig confirmed the clinical observation that heat may cause a severe disturbance of the dermal blood vessels in both pig and man without causing recognizable damage to the epidermis. The capillary loops of the dermal papillae became dilated and elongated and filled with closely packed masses of erythrocytes. Separation of collagen fibers by edema fluid was obvious and perivascular mantles of extravasated erythrocytes were often seen. The escape and extravascular deterioration of erythrocytes in such a lesion was often sufficient to result in brown discoloration of the target area for as long as a week. Extravascular fibrin was not encountered nor did collagen fibrils appear to be swollen. Between 12 and 24 hours after such an injury was sustained, occasional polymorphonuclear leucocytes were found in the edema fluid. Neither thrombosis nor visible alteration in the vascular endothelium was seen, despite the fact that superficial vessels were filled by static, sausagelike agglomerates of red blood cells.

REVERSIBLE IMPAIRMENT OF EPIDERMAL ANCHORAGE

During most, and possibly all, injurious episodes of cutaneous hyperthermia in which the temperature of the dermis was maintained for a sufficient time at 49 C or higher, there was a brief interval subjacent to the threshold for transepidermal necrosis in which the adhesion of epidermis to dermis was impaired.

The attainment of this degree of injury was recognized by the ease with which the epidermis could be dislodged by friction. If the exposure was discontinued before further injury was sustained and if the loosened epidermis was not dislodged either by trauma or vesication, the change was often reversible in the case of the pig and after 12 or 18 hours the original firm anchorage of the epidermis was usually regained. Unless the exposure had been excessive, such injuries subsided without further evidence of cell death.

If skin altered in this manner was protected against mechanical artifact, there was no microscopic evidence either in the basal epithelial cells or in the underlying dermis by which impairment of the epidermal anchorage could be recognized. If, however, sufficient friction was applied to the temporarily insecure porcine epidermis to cause its detachment, microscopic examination revealed a fringe of up-rooted or fractured tonofibrils protruding from the lower ends of the detached basal epithelial cells. The protruding fibrils appeared to have been pulled out of their anchorage in the superficial dermal felt work of collagen fibers. It was not determined whether the essential change responsible for such epidermal instability was a deterioration of the extracellular extensions of the tonofibrils which predisposed them to rupture or a softening of the dermal collagen in which they were embedded. The latter was considered the more plausible explanation of the phenomenon. In man it is doubtful that the tonofibrils of the epidermal cells have much if anything to do with the attachment of epidermis to dermis. In human skin, the epidermis appeared to be cemented to, rather than rooted in, the dermal collagen.

It has already been indicated that when porcine skin sustained this type of cutaneous burn, recovery sometimes took place within 24 hours without death of cells, providing the damaged area was protected against mechanical disturbance during that period when its anchorage to the dermis was insecure.

Too few appropriate specimens of human burns were available for microscopic examination to permit conclusions regarding the threshold at or the fre-

quency with which this particular type of first-degree thermal injury occurs in man. The opinion was gained from clinical observations of human burns that thermal exposures insufficient to cause primary epidermal necrosis may result in a temporary impairment in the adhesion between epidermis and dermis. If such a temporarily insecure layer of epidermis is detached by friction or vesication, the detached cells would undoubtedly die. Thus, it is entirely possible that the phenomenon of vesication results, in some instances, in secondary destruction of human epithelial cells that would otherwise survive. If this be true, and if the thermal exposure has been insufficient to cause primary transepidermal necrosis, the immediate institution of pressure to prevent epidermal displacement by vesication should predispose to early and uncomplicated healing of what might otherwise become an open lesion.

IRREVERSIBLE THERMAL INJURY OF EPIDERMAL CELLS

Material for microscopic study was available from almost every conceivable kind, grade, and stage of thermal injury of the skin of the pig. Although a wide range of experimental thermal injuries of human skin was studied clinically, most of the burns that were available for microscopic examination were obtained from autopsies. Thus, there was no direct information regarding the intensity or duration of the thermal exposures that were responsible for most of the burns of human skin that were studied microscopically. The impression was gained, however, that, apart from the phenomenon of vesication, the cytological changes induced by heat in the epidermis of man were similar, if not identical, to those observed in the pig (see Figures 17 to 22). Attention has already been directed to the fact that the time-temperature threshold for the destruction of epidermis is almost identical in human and porcine skin (see Figure 14).

The first manifestation of irreversible thermal injury of the epidermis was a change in the distribution of water and solids within the nuclei of the cells of the intermediate zone. As the nuclei swelled, their chromatin granules coalesced to form compact crescentic masses immediately beneath and attached to one side of the nuclear membranes (Figure 20). When the swollen nucleus ruptured, the peripherally distributed chromatin contracted to form a dense and irregularly shaped central mass which remained separated from the surrounding cytoplasm by clear fluid.

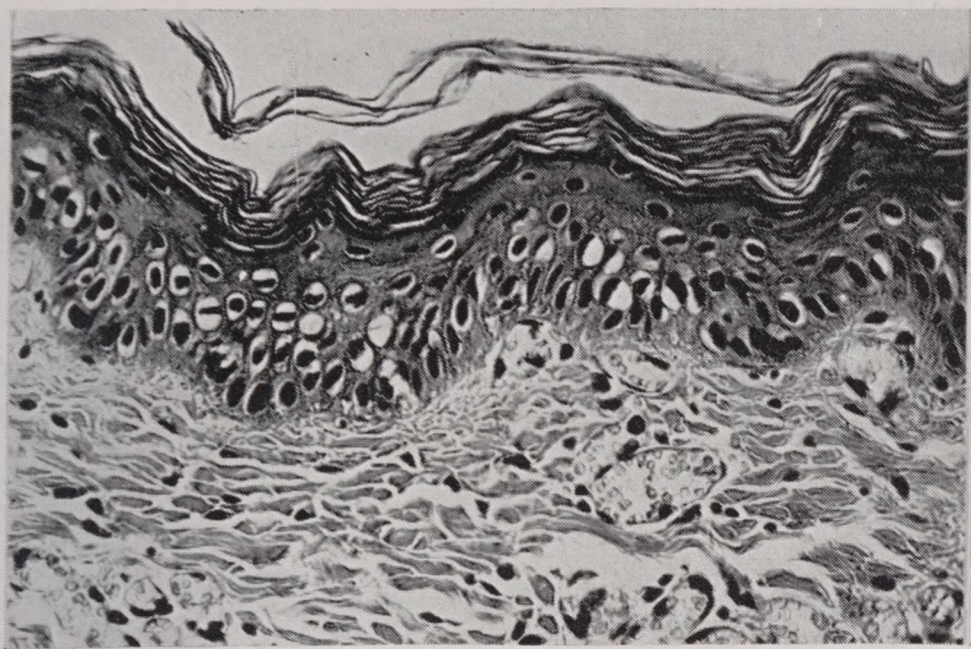


FIGURE 17

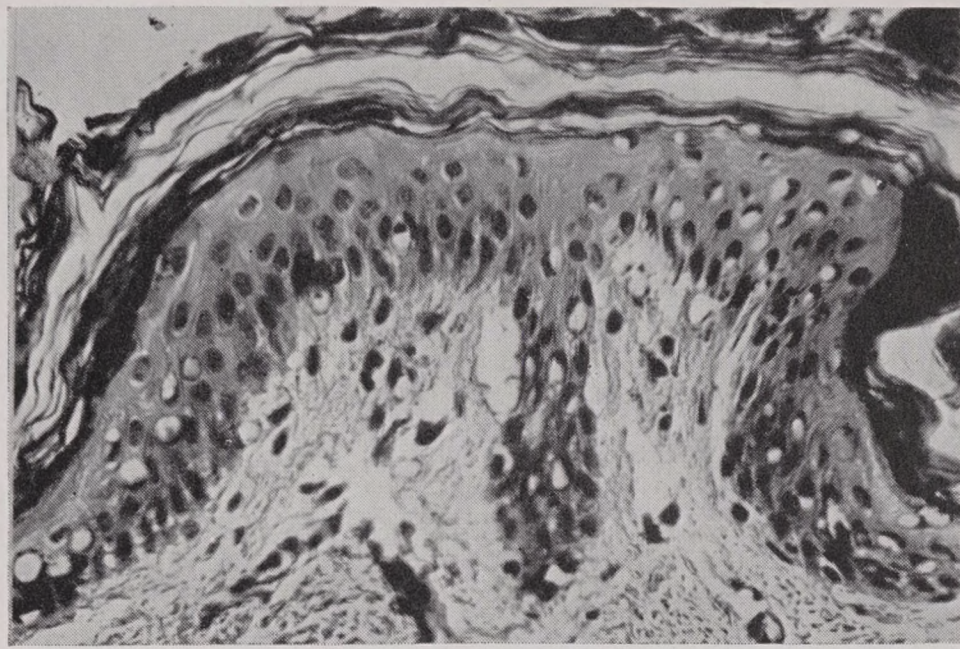


FIGURE 18

Photographs of severe first-degree burns of porcine (Figure 17) and human (Figure 18) skin showing degenerative changes in epidermis. In Figure 17, there is generalized pyknosis of nuclei and it is not likely that any epidermal cells included in picture would have recovered. In Figure 18, changes are focal rather than general and most of altered nuclei are swollen and show peripheral displacement of chromatin. This type of nuclear change precedes that shown in Figure 17. Both specimens were excised within an hour after injury was sustained. In both instances, epidermal attachment to dermis was insecure and lesion shown in Figure 18 would probably have gone on to vesication in normal course of events. Magnification 400 $\times$.

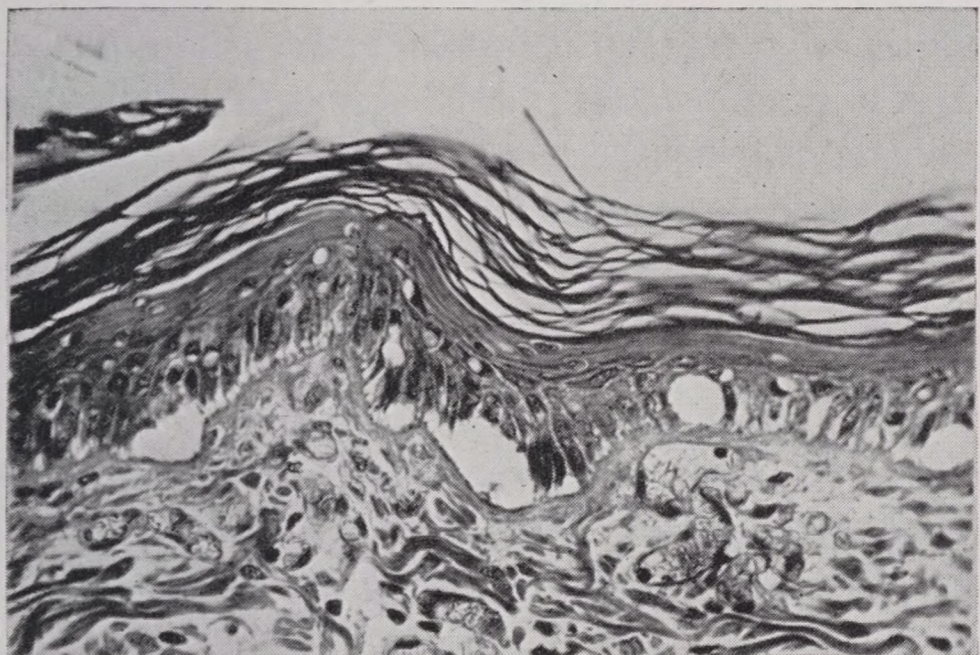


FIGURE 19

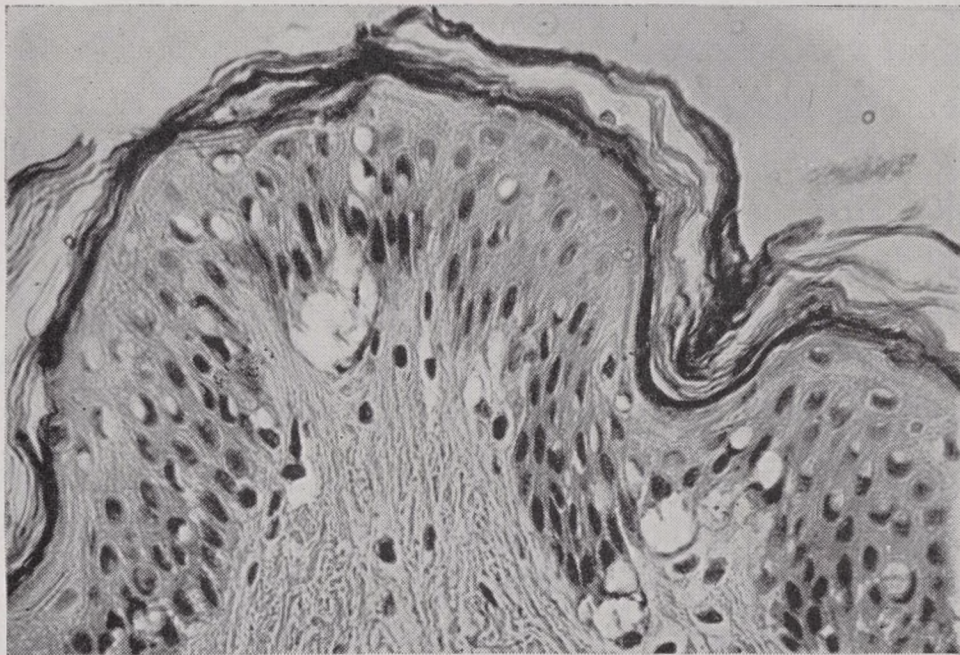


FIGURE 20

Photographs of early second-degree burns of porcine (Figure 19) and human (Figure 20) skin showing early spontaneous detachment of epidermis from dermis. Vacuolar cytoplasmic disintegration of basal cell layer has been added to nuclear changes similar to those shown in Figures 17 and 18. In Figure 19, tonofibrils that were uprooted from their anchorage in dermis can be seen projecting from detached basal cells. Magnification 400 $\times$.

This fluid, whether extruded into the nuclear lacuna or contained within the distended nuclear membrane, was faintly basophilic and sometimes contained a few fine Feulgen-positive fragments of chromatin.

Pyknosis of nuclei was by no means pathognomonic of thermal injury. Spontaneous nuclear pyknosis was sometimes seen in the upper zone of normal unheated epidermis and was caused by injuries other than heat.

In the case of subthreshold exposures sufficient to injure the upper layers of epidermal cells but insufficient to cause transepidermal necrosis, the types of

nuclear change which have been described were frequently focal and difficult to distinguish from qualitatively similar changes in control material. Even though it could be plausibly assumed that all of the cells at a given level were exposed to the same degree of hyperthermia, it was not uncommon to find groups of cells with normal appearing nucleuses interspersed among those that showed advanced degenerative change (Figure 18). The reason for this apparent difference in the susceptibility of cells in the same layer to heat was not apparent.

If the thermal exposure was of sufficient intensity

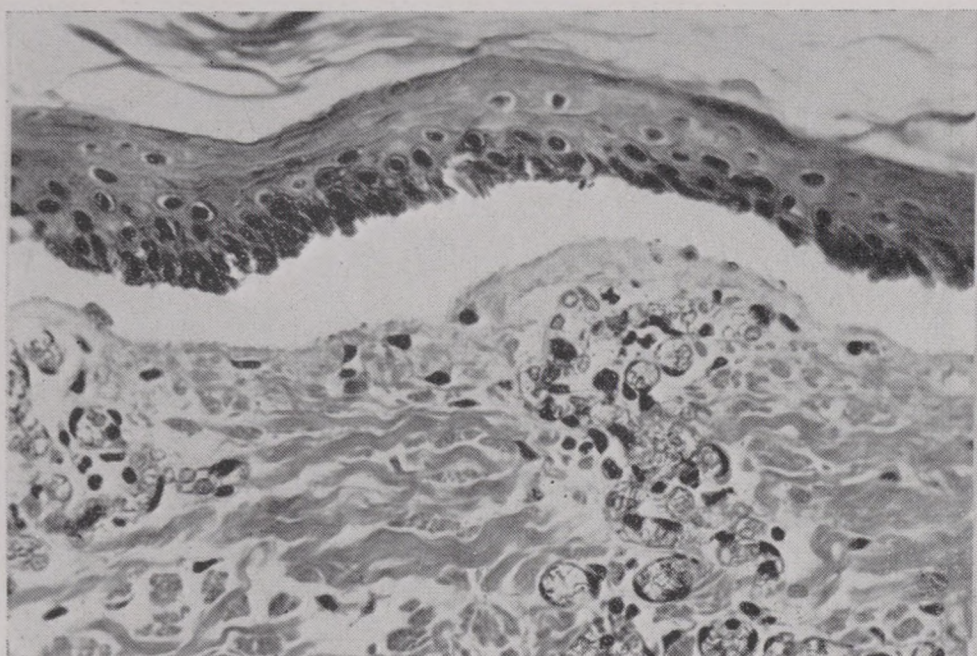


FIGURE 21

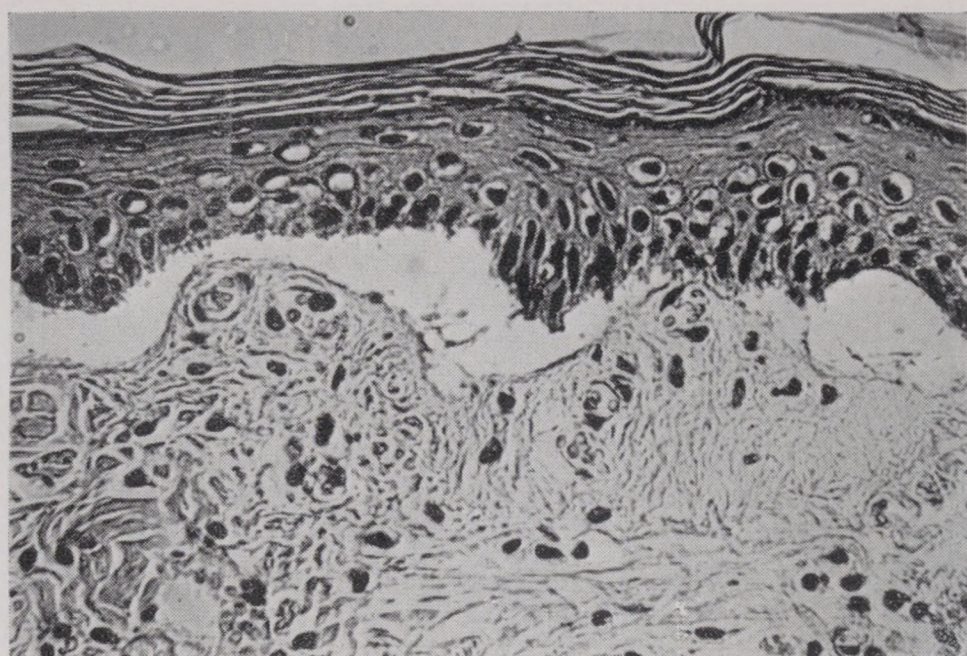


FIGURE 22

Photograph of pseudo-vesicle of porcine skin (Figure 21) and early true vesicle of human skin (Figure 22). In each, transepidermal necrosis appears to be complete. In porcine skin, detached epidermis would have remained *in situ* as flaccid membrane. In human skin, detached epidermis would have been elevated by collection of edema fluid between it and dermis. Magnification 400X.

or duration to cause irreversible cellular injury, nuclear changes of the kinds described in the foregoing paragraphs were usually apparent immediately after the conclusion of the episode of hyperthermia. This was not invariably the case, however, and after certain exposures at relatively low temperature (under 47 C) a postexposure interval of between 6 and 12 hours was sometimes required for the development of recognizable nuclear alterations. Moreover, if the exposure was of sufficient severity to cause pseudovesication in the pig or true vesication in man, many of the nuclei which were apparently undamaged at the conclusion of the exposure disintegrated during the next 24 hours.

If the episode of hyperthermia was such as to cause visible alterations in nuclear structure, there was inhibition of mitotic division throughout the entire area of exposure for many hours. No evidence was derived from the microscopic study of subthreshold exposures to indicate that hyperthermia predisposed to acceleration of mitotic activity. The impression was gained that nuclear swelling with coalescence of chromatin granules constituted evidence of an irreversible cellular change and invariably led to premature death and desquamation of the altered cells.

In the pig, the irreversibly damaged epidermal cells were gradually desquamated over a period of a week or 10 days in the form of thin brown scales.

Alterations in the appearance of nuclei in the upper and intermediate layers of epithelium were thought to provide the earliest morphological evidence of primary thermal injury of the epidermis and were

frequently encountered without perceptible damage to the cells of the basal layer. Characteristically, the earliest change in the basal cell layer caused by hyperthermia was cytoplasmic rather than nuclear. The injured basal cells swelled and their cytoplasm became vacuolated and increasingly acidophilic (Figures 19 and 20). The vacuolization appeared to be due in part to imbibition of fluid and in part to redistribution of water and solids within the cells.

The fluid contained within the cytoplasmic vacuoles was clear, nonsudanophilic, and sometimes contained a delicate mesh of granular amphophilic debris. With severe injury there was widespread rupture and disintegration of the lower ends of the basal cells.

17.7.4 Second-Degree Reactions

TRANSEPIDERMAL NECROSIS

The time-temperature characteristics of exposures just sufficient to cause transepidermal necrosis in both man and pig are indicated in Figure 14. In man, whether or not a thermal exposure has destroyed the epidermis can usually be determined by the occurrence or nonoccurrence of vesication within the first 24 hours. To recognize with certainty during the first day or two after a near-threshold exposure whether or not porcine epidermis has been destroyed, the skin must be excised and examined microscopically. When the area of injury was 7 mm in diameter and when the duration and intensity of the exposure was at or not far above the threshold required for transepidermal necrosis, the time usually required for complete healing was between 5 and 10 days in the pig and between 1 and 2 weeks in man.

In the pig, microscopic evidence that an exposure had been sufficient to cause transepidermal necrosis was provided by the changes that had occurred at the basal cell level. With the disintegration of the cytoplasm of the proximal or lower ends of the injured basal cells, there was at first focal and later extensive spontaneous detachment of the epidermis from the dermis. In the pig, the amount of fluid that collected beneath the loosened epidermis was never sufficient to produce true vesication.

With still more severe hyperthermia, the cytoplasmic disintegration of the basal cells was followed by nuclear changes similar to those seen in the more superficially located cells. If the epidermal detachment was incomplete, stretching and attenuation of the remaining bridging cells and their nuclei was often seen. Such attenuated masses of chromatin were often stretched to two to three times the original length of the entire cell.

In the event that the surface temperature of the epidermis was brought rapidly to a level of 55 C or higher and maintained at that level for a period longer than that necessary to cause cell death, transepidermal coagulation was likely to occur so quickly as to inhibit recognizable redistribution of intra- and extracellular fluids. In such an event, neither the cytoplasm nor the nucleuses of the epithelial cells appeared swollen (Figure 25). On microscopic examination, both appeared shrunken, the former being intensely and uniformly acidophilic and the latter small and homogeneously basophilic.

VESICATION

Attention has already been called to the fact that a common effect of heat on the skin of both man and pig is impairment of the attachment of the epidermis (Figures 21 and 22) to the dermis, and the opinion expressed that this may be due either to a change in the physical state of the superficial dermal collagen or to disruption of the basal layer of epithelial cells. A common collateral effect of cutaneous hyperthermia, and one that is essential to true vesication, is an outpouring of fluid from the dermal capillaries.

When a thermal exposure of human skin was sufficient to impair the attachment of the epidermis, the amount of edema fluid that collected between it and the dermis was usually sufficient to elevate and stretch the entire layer of dead, dying, and living cells and to form a vesicle. Although vesication of human skin was usually an almost immediate response to a thermal exposure of sufficient severity to

cause primary epidermal injury, there were several circumstances in which it was either delayed or inhibited.

Delayed vesication was most frequently seen after long-time exposures at low temperatures or after flash exposures at high temperatures. In both circumstances it seemed likely that the delay was due to the fact that the vascular damage was relatively mild, and that hours rather than minutes were required for enough fluid to collect beneath the damaged epidermis to form a vesicle.

Another circumstance in which vesication was delayed or prevented was when the injury was so overwhelming that the dermis and its superficial capillaries were almost immediately coagulated. With such thermal injury, the level at which edema developed was too deep to result in vesication.

Thus, in man, the nonoccurrence of vesication after a thermal exposure sufficient to cause severe injury of the epidermis may mean that the dermal hyperthermia was either inadequate to result in edema or that it was so overwhelming that the superficial capillaries were almost immediately occluded.

In no instance was true vesication of porcine skin observed. This was true despite the fact that many of the injuries met at least two prerequisites to vesicle formation: namely, sufficient vascular injury to result in dermal edema and sufficient impairment of the adhesion between epidermis and dermis to permit easy mechanical detachment of the former (Figure 21). Failure of the pig to vesicate appeared to be due to the fact that the amount of edema fluid that penetrated the surface of the dermis was never sufficient to elevate the epidermis. In the absence of evidence to the contrary, a tenable explanation for nonvesication in the pig is that an episode of hyperthermia that is sufficient to impair the attachment of the epidermis to the dermis characteristically alters either the superficial felt work of dermal collagen fibers or the walls of the capillaries contained by it in such a way that they become virtually impermeable to edema fluid.

The nature or, for that matter, the existence of this theoretical alteration in the permeability of the collagen or the capillary walls was not disclosed by microscopic examination. When the severity of an exposure was considerably in excess of that required to destroy the epidermis, swelling of the superficial dermal collagen and occlusion of its capillaries could be recognized. There was, however, a wide range of exposures between the threshold for epidermal ne-

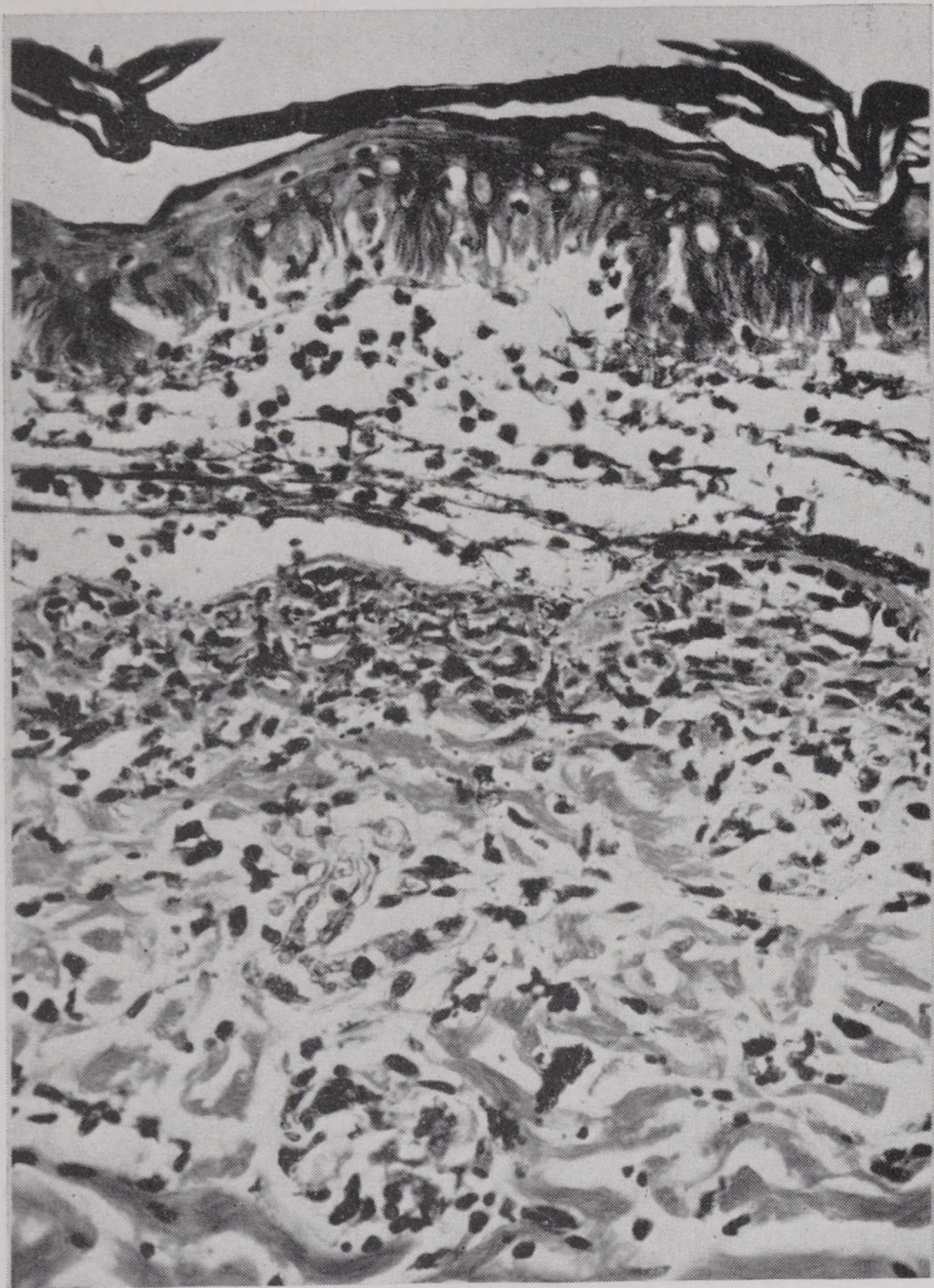


FIGURE 23

Photographs of mild (Figure 23) and moderately severe (Figure 24) third-degree thermal reactions in porcine epidermis 24 hours after injury. Both injuries were produced by episodes of hyperthermia that were of low intensity (under 55 C) and long duration. In both instances, irreversibly injured dermal tissue will undergo autolysis and organization. Magnification 400X.

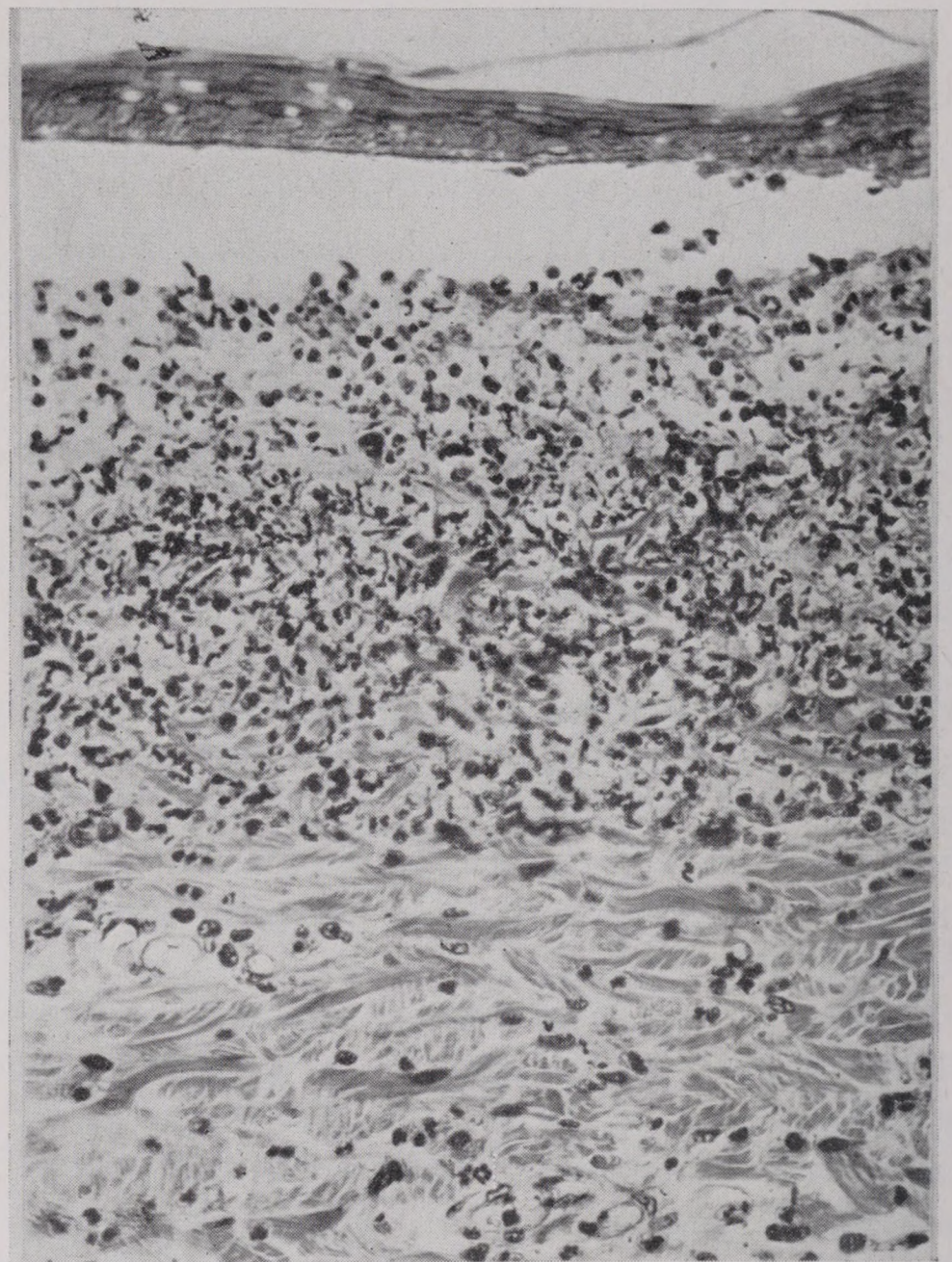


FIGURE 24

crosis and that for recognizable swelling of collagen or occlusion of capillaries in which the microscopic examination of the pig's skin disclosed no explanation for the failure of porcine skin to vesicate.

17.7.5 Third-Degree Reactions

The more a thermal exposure exceeded the threshold required for destruction of the epidermis, either in respect to temperature or time, the deeper the injury and the longer the recovery period necessary for repair and regeneration. In both pig and man, several weeks represented the minimum healing time if a significant degree of dermal injury had been sustained.

FURTHER CHANGES IN EPIDERMIS

In the case of the pig, prolonged exposure at a relatively low surface temperature (under 55 C) caused relatively little additional change in the microscopic appearance of the epidermis. In the higher range of surface temperatures, significant prolongation of the

rate of duration of exposure beyond the time necessary to destroy the epidermis modified the quality of the superficial changes both in human and porcine skin. In man, vesication was permanently inhibited and in both man and pig the loosened epidermis became reattached to the damaged dermis. Early solidification and contraction, both cytoplasmic and nuclear, occurred before there was opportunity for the development of the retrogressive changes observed in first- and second-degree reactions. The higher the temperature, the shorter the time required to cause transepidermal coagulation. With exposures to superheated air, desiccation was superimposed on the effects of heat, and, soon after the temperature rose above 300 C, carbonization of the dry tissue began to take place.

RED AND PALE BURNS

The surface color of third-degree burns ranged from pale gray through red, purple, and brown to



FIGURE 25. Third-degree thermal reaction in porcine skin showing coagulation of epidermis and dermis 24 hours after injury. Bundles of denatured dermal collagen appear swollen and homogeneous and become increasingly basophilic. Thermal reactions of this type were encountered only where surface temperature had been brought to and maintained at level of 55 C or higher. Magnification 400X.

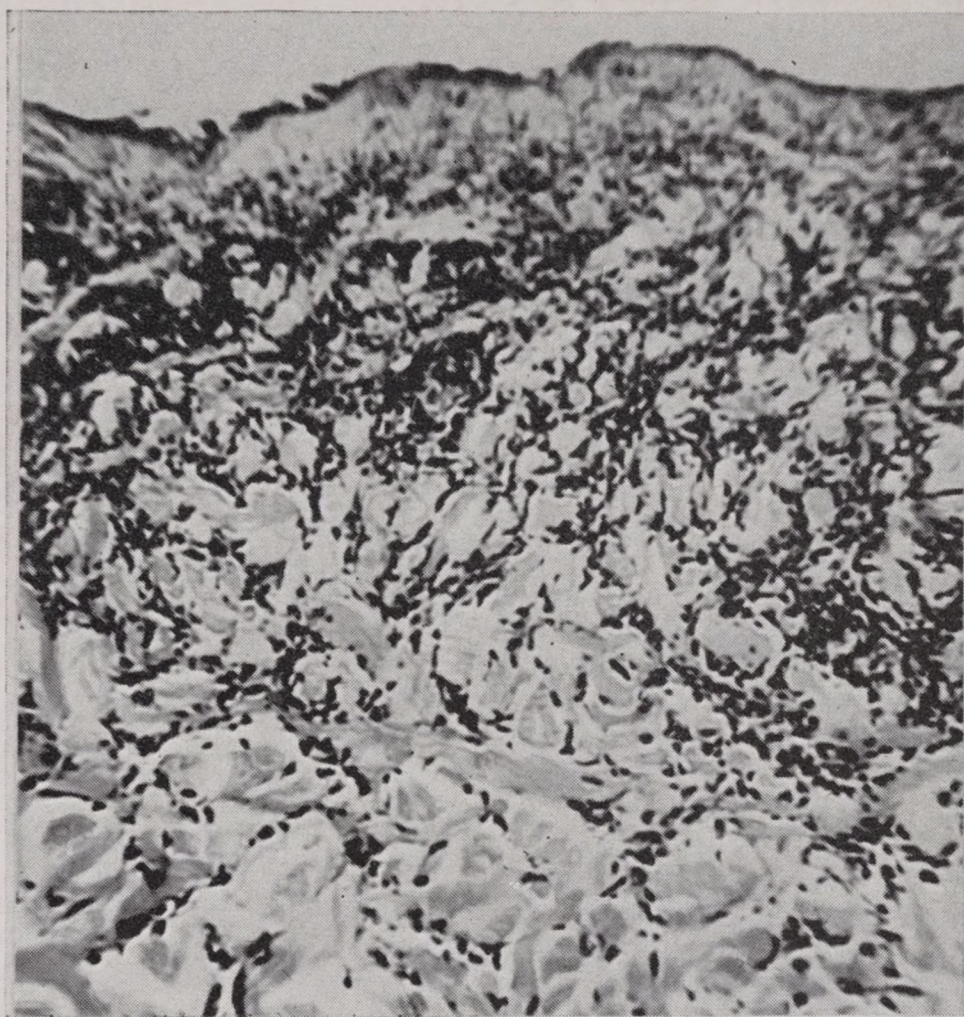


FIGURE 26. Photograph of third-degree thermal reaction in porcine skin 72 hours after injury. Exudative cells have migrated into interstices between bundles of coagulated collagen. Precise level at which this injury will be stabilized is not yet apparent. Healing will be slow because of resistance of denatured collagen to autolysis and organization. Magnification 400X.

black, depending on certain attributes of the exposures responsible for their production.

A black or carbonized surface resulted from exposures at temperatures in excess of 200 C (Figure 28). The precise temperature at which carbonization began was not determined.

A red, purple, or brown surface, due to the presence of blood in the superficial layer of the skin, resulted from exposures in which the dermal temperature was raised slowly enough to permit advanced engorgement of the superficial capillary plexus before the occurrence of coagulation.

A gray or ischemic surface indicated that the upper portion of the dermis had undergone thermal coagulation before the superficial capillaries had become fully engorged.

The reciprocal relationships of time and temperature as they relate to the visibility of hemoglobin beneath the surface of a third-degree thermal reaction is shown in Table 10. It was found that at atmospheric pressure and at surface temperatures of 65 C or lower burns appeared superficially hyperemic regardless of the duration of exposure. When a 70 C surface exposure was interrupted at the end of 2 seconds, the lesion remained red, but, if it were pro-

longed to 3 minutes, the zone of reactive hyperemia became overlaid by so thick a layer of coagulated tissue that it was no longer visible. Above 70 C all exposures of a second or longer coagulated the superficial plexus of dermal capillaries so rapidly that most or all of the blood contained in them was displaced to a level too deep to be seen from the surface.

POOLING OF BLOOD IN HYPEREMIC BURNS

A qualitative impression of the pooling of blood in the dilated cutaneous vessels after an injurious episode of hyperthermia was derived from the photomicrographs shown in Figure 11. In order to learn something of the actual amount of blood that was present in such lesions, samples of both normal and hyperemic skin were excised for chemical examination. Samples of skin and subcutaneous tissue having an area of 25 cm² and extending to the deep fascia were taken from the lateral thoracic area of each of nine pigs and their iron content was determined. Two of the samples represented normal skin and the other three were from areas of hyperemic burning.

It is apparent from the results of the experiments shown in Table 16 that a relatively large proportion

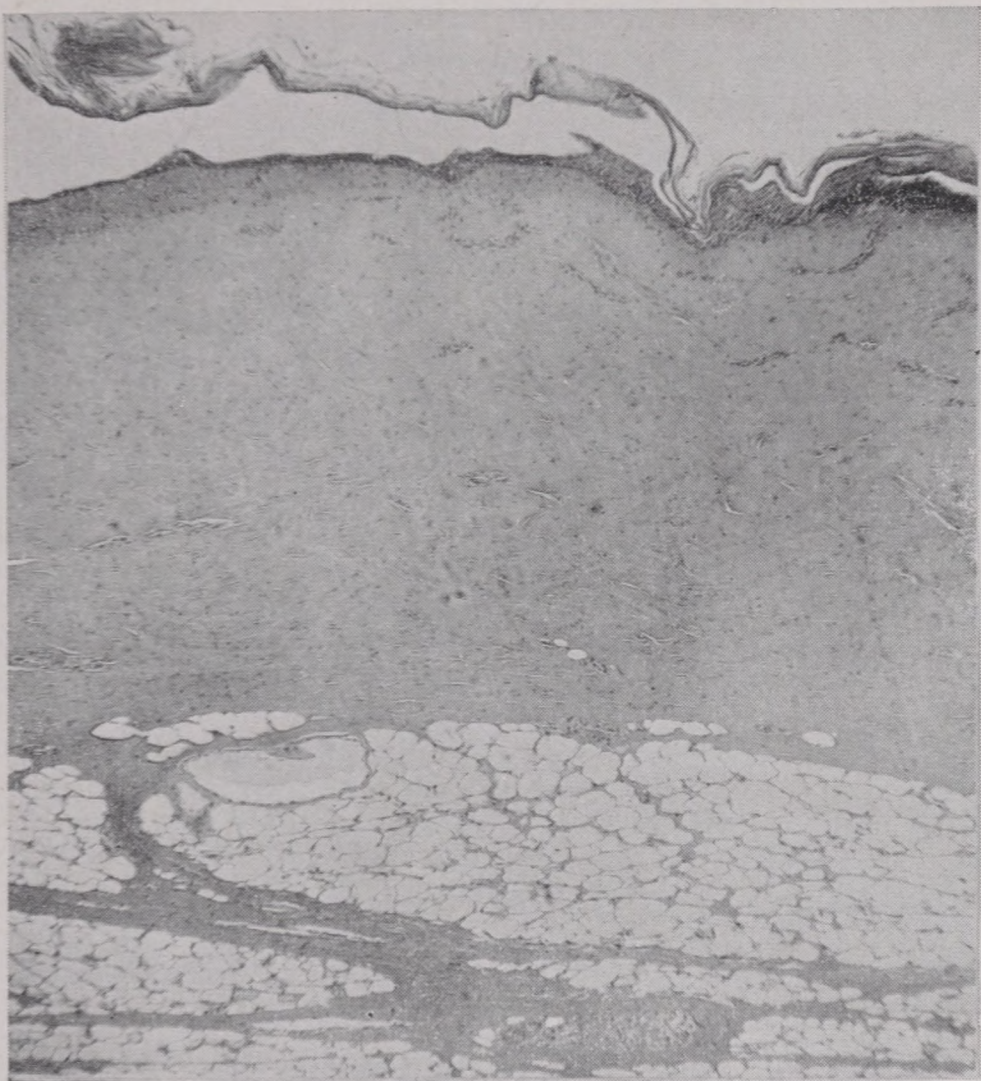


FIGURE 27. Transcutaneous coagulation resulting in deep ischemic burn. Five-minute exposure to air at 200 C. Surface temperature of skin not known but probably in excess of 55 C. Epidermis has become re-attached to dermis. Magnification 85X.

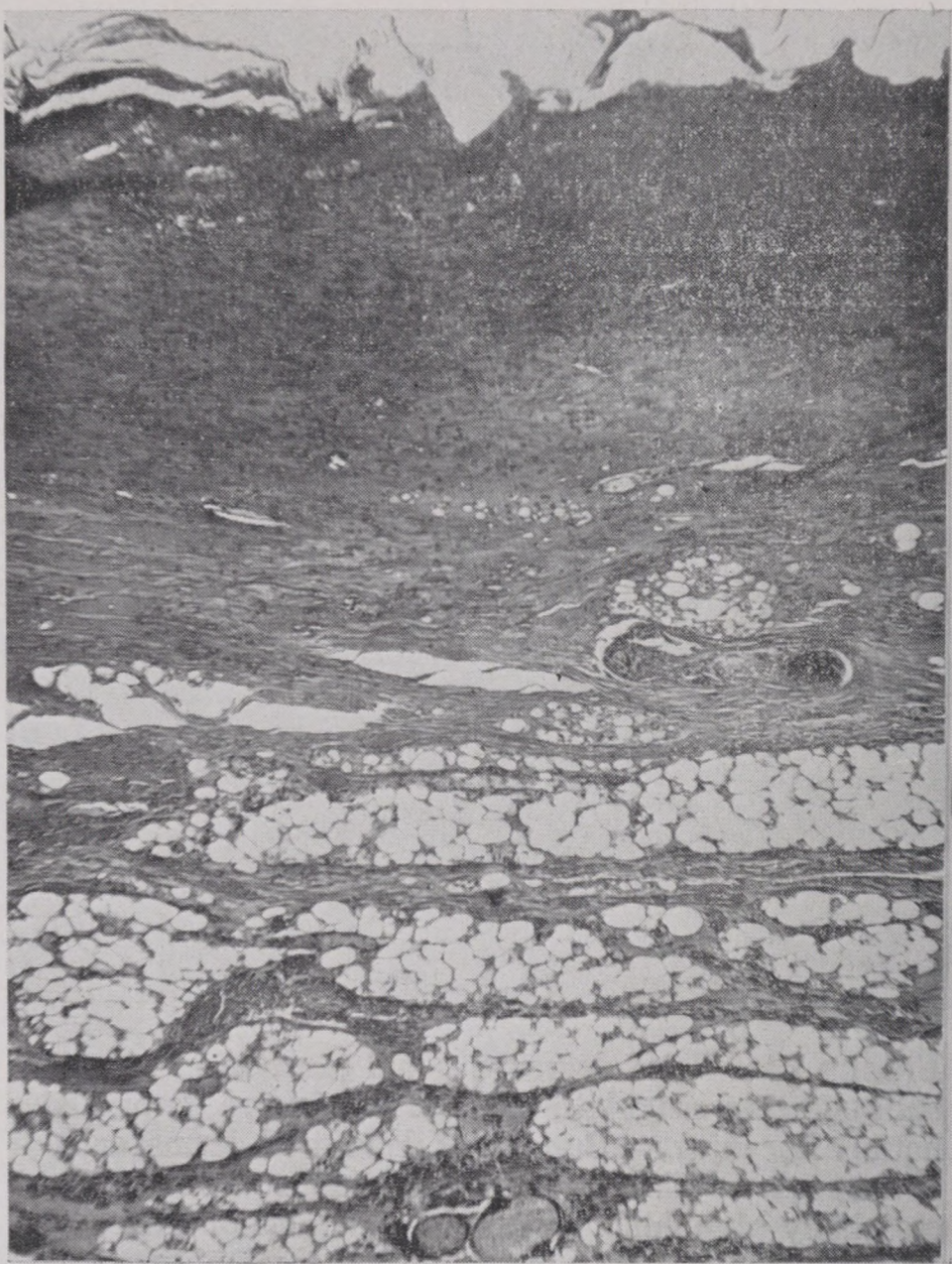


FIGURE 28. Carbonization of surface and intense basophilia of coagulated dermis due to 2.5-minute exposure of skin at 405 C. Effects of ambient heat augmented by radiant energy. Magnification 85X.

of the total circulating blood of an animal may be pooled in the skin and subcutaneous tissue as a result of thermal injury. Calculations based on the amount of recoverable iron per unit of surface area

TABLE 16. Pooling of blood in the subcutaneous vessels due to thermal injury.

Condition of skin	Mg iron in 25 cm ² sample	Est. cc of blood in 25 cm ² sample
Normal	0.06	0.11
	0.10	0.18
Moderate hyperemia	0.14	0.25
	0.29	0.51
Severe hyperemia	0.56	1.00
	0.60	1.10
	0.40	0.70
	0.39	0.70
	0.37	0.67

of burned skin in relation to the body weight indicated that as much as 30 per cent of the erythrocytes of an animal suffering from generalized cutaneous hyperemia could be accounted for in the skin and subcutaneous fat.

EFFECT OF COMPRESSIVE HYPERTHERMIA ON COLOR OF A BURN

In a preceding section attention was called to the fact that compression of the skin surface during ex-

posure to heat did not increase the vulnerability of the epidermis to thermal injury. It was found, however, that compression of the skin was capable of modifying the superficial color of the burn even though there was no quantitative increase in its severity. To determine the circumstances in which compression of the skin during an episode of hyperthermia may modify subsequent surface color of the lesion, the experiments summarized in Table 17 were undertaken. In some, hot water was applied at atmospheric pressure; in others, it was applied with a compressive force of 120 mm of mercury.

The results of these exposures indicated that the color of burns resulting from surface temperatures lower than 55 C was not affected by pressure, but that an increase in pressure during exposures at surface temperatures of 60 C or higher determined whether the surface of the resulting burn would be ischemic or hyperemic. Thus, an exposure at atmospheric pressure at 60 C produced a red burn even though it was extended for as long as 5 minutes. With increase in pressure, a 2-minute exposure at the same temperature resulted in a pale burn and yet the

TABLE 17. Experiments to determine the circumstances in which compression of the skin during an episode of hyperthermia may modify subsequent surface color of the lesion.

Temp of surface (C)	Duration of hyperthermia (seconds)	Pressure on skin (mm Hg)	External appearance of burn 24 hours after exposure	
			Ischemic	Hyperemic
70	5	0	..	+
	5	120	+	..
65	30	0	..	+
	30	120	..	+
	60	120	+	..
	1,200	0	..	+
60	60	0	..	+
	60	120	..	+
	120	120	+	..
	300	0	..	+
55	1,800	0	..	+
	1,800	120	..	+

depth to which the tissue had been destroyed in the latter was less than that to which it had been destroyed in the former.

At 70 C a 5-second exposure at atmospheric pressure resulted in a red burn, but, with an additional pressure of 120 mm of mercury, the resulting burn appeared ischemic.

Microscopic examination of these lesions provided evidence that the color of a burn was not a reliable criterion by which to judge its depth. After hyperthermic episodes of comparable duration and at the same surface pressure, a red surface color usually indicated that the lesion was less severe than one having a gray surface. Without knowledge of time, temperature, or surface pressure during the period of exposure, it is not possible to estimate the relative severity of burns on a basis of surface color.

OTHER EFFECTS OF HEAT ON DERMIS

After edema and pericapillary extravasation of erythrocytes the earliest recognizable extravascular alteration of the dermis was swelling of the collagen fibers. This occurred first in its most superficial layer where, in the case of porcine skin, the projecting tonofibrils of the basal epithelial cells were imbedded in the collagen of the subjacent connective tissue.

As the intensity and duration of the hyperthermia increased, the corium tended to lose its fibrillar character and was converted into a thin lamella of homogeneous acidophilic material as though its individual fibers had been converted to a gel. With increasing exposure the swelling of collagen became apparent at greater and greater depths in the underlying connective tissue (Figure 25). Expansion of collagen

bundles tended to collapse the dermal blood vessels and the loose areolar tissue that surrounded them. Visible edema receded in advance of this type of alteration as though the fluid were imbibed or displaced by the denatured collagen. Not until 24 or 48 hours had elapsed was it possible by microscopic examination to recognize the line of demarcation between reversible and irreversible dermal injury (Figures 23 and 24).

From the intact blood vessels of the deeper and relatively uninjured tissues, leucocytes migrated upward through the perivascular interstices and into the zone of denatured collagen. A frontier was eventually established between the tissue capable of regeneration and repair and that destined to be sequestered in the form of a desiccated crust. The deeper the lesion, the longer the time required for the stabilization of such a frontier. The transition between the obviously necrotic tissue of the upper dermis and the least disturbed tissue of the deepest portion of the zone of hyperthermia was a gradual one. Exudation of leucocytes occurred within a few hours and within 24 hours usually served to delineate the zone within which the plane of irreversible injury would eventually become stabilized. Within 2 or 3 days fibroblasts and new capillaries began to push up toward the surface in the interfascicular interstices of the denatured collagen. The least affected connective tissue at the base of the reaction zone recovered quickly and without apparent loss of fixed tissue cells. The fate of the more severely injured collagen varied according to the extent to which it had been denatured. Thermal denaturation of collagen at temperature levels under 55 C did not usually result in the kind of coagulative change that made the collagen resistant to subsequent autolysis and organization (Figure 26). Collagenous denaturation at temperatures over 55 C often resulted in an irreversible type of coagulation which resisted lysis and eventually led to sequestration en masse. Thus, deep and severe burns resulting from surface exposures lower than 55 C were likely to remain soft and red and the necrotic tissue was susceptible to organization. Deep burns resulting from higher temperatures were characteristically firm and pale and the necrotic tissue was sequestered rather than organized. After exposures to temperatures between these two extremes the dead and damaged connective tissue was infiltrated by leucocytes and penetrated by granulation tissue and its necrotic elements were gradually resorbed and replaced by new connective tissue.

During the time required to establish the level of irreversible injury, tentative tongue-like masses of new epithelial cells grew out from the margins of the lesion and from the viable roots of partially destroyed hair follicles as though they were seeking a sufficiently well-stabilized layer of connective tissue to provide support and nutrition. Repeated crops of such new epithelial cells extended over or into the granulation tissue and failed to survive, for reasons not disclosed by microscopic examination.

The number of experimentally produced deep burns of human skin was not great enough to draw any definitive conclusion regarding the relative rates of healing of such lesions in man and pig. The impression was gained, however, that lesions of similar area and depth heal more rapidly in the pig.

17.7.6 Summary

Comparison of effects of heat on human and porcine skin: In a previous section of this chapter it was shown that the quantitative relationships between temperature, duration of hyperthermia, and depth of injury were similar in human and porcine skin. In this section it has been shown that there is a striking qualitative similarity between the microscopic alterations that are caused in human and porcine skin by hyperthermia. The most important qualitative difference is that true vesication was not observed in the pig, whereas in man it is a characteristic cutaneous reaction to certain types of thermal injury. The reason for this difference has been discussed. Attention was called to the fact that vesication is an undesirable phenomenon in that it may result in the separation and death of viable epidermal cells and that there is reason to believe that healing of certain burns in man would be hastened if vesication could be prevented.

Sequence of changes caused by harmful episodes of hyperthermia: The earliest changes are latent, in the sense that they are not associated with visible alteration in the appearance of the damaged cells. Such changes are reversible.

Beyond the stage of latent injury, the pathological changes produced by exposure to heat are of two kinds: those that represent the reaction of living tissue to injury and those that represent the effects of excessive heat on cells and intercellular substances that have already sustained irreversible injury. The former may or may not be reversible and differ in nature according to the type of cell or tissue in which the reaction has occurred. The latter are of impor-

tance principally with respect to the extent to which such postvital thermal denaturation interferes with the organization and disposal of the necrotic tissue. Both types of reactions have been described in detail.

17.8 CONSIDERATION OF THE NATURE OF PHYSICAL AND CHEMICAL CHANGES INDUCED IN TISSUE BY HYPERTHERMIA[†]

17.8.1 Introduction

Ideally, an attempt to elucidate the precise nature of the changes produced by heat on the skin should be based on a knowledge of the various physical and chemical phenomena that are normally essential to the survival and functional integrity of the living cells that comprise cutaneous tissue. If it were then possible to observe the alterations of each of these physical and chemical functions with temperature, a direct solution of the problem of how heat injures the skin might be reached. Unfortunately, detailed information regarding the basic physical and chemical properties of the skin or the effects of temperature thereon does not exist. In fact, very little qualitative and almost no quantitative data are available on even the general physical and chemical attributes of skin constituents as of to date. It is apparent, then, that any consideration of temperature-induced physical and chemical changes which may lead to thermal death must be based on the known *in vitro* effects of temperature on substances that are akin in function and/or properties to those which probably occur in cutaneous tissues.

Since nearly all the quantitative experimental data derived from this investigation deal with epidermis, the ensuing discussion will be limited primarily to this tissue. In Sections 17.6.5 and 17.9.3, these data are shown to be quantitatively predictable by the standard form of a rate equation,¹⁹ specifically, equation (7). In this equation there appear two empirical and experimentally determinable constants, namely, A and ΔE ; any theoretical consideration of the cause of thermally induced transepidermal necrosis should take into account, at least qualitatively, the numerical values of these quantities. Aside from certain general conclusions regarding the entropy of the overall process,²⁰ little specific information can be obtained from the numerical value of A , since this constant is intimately connected with the as yet un-

[†] By F. C. Henriques, Jr.

known detailed physical and chemical properties and functions of the epidermal constituents. This is not the case, however, with ΔE , and thus, before proceeding further, a brief general consideration of the nature of ΔE , the activation energy in calories per mole, is in order.

17.8.2 Thermal Injury and Energy of Activation²⁰

In general, the kinetics of any given physical and/or chemical process depends upon the total energy content of the constituents involved. If this energy content is less than a certain critical value, known as the activation energy, the process cannot take place; if the energy content is equal to or greater than this critical value, the process may take place. Thus the rate of the process will be proportional to the fraction of these constituents which, collectively considered, possess an energy content at least equal to the activation energy. This fraction is deduced from the Maxwell-Boltzmann energy distribution law, which states that

$$f = e^{-\Delta E/R(T+273)} \quad (16)$$

where f is this fraction, and the remaining symbols have been previously defined. Equation (16) determines only the temperature coefficient of a rate process, since, as shown by equation (7), the rate of a process is also proportional to one other factor that is essentially nondependent on temperature, namely A .

Thus, the rate of any conceivable process that may result in cell death, whatever it may be, depends upon a critical energy content of the participants. The fraction of the participants, collectively considered, having this energy is determined by the activation energy and the temperature [equation (16)]. The availability of this fraction is requisite but not in itself sufficient to allow the process to proceed.

An inspection of equation (16) shows that the temperature coefficient of any kinetic process is a strong function of the activation energy; for example, in the neighborhood of 50 C, the rate of a process with an activation of 1, 10, or 100 kcal/mole will be altered by about 0.4 per cent, 7 per cent, or 70 per cent, respectively, per unit change in temperature in C.

The kinetics of a considerable number of physical and chemical phenomena have been studied in detail and it is possible to classify all rate processes and, hence, in particular, those mechanisms which may be

of considerable importance in the general consideration of thermal injury, according to the order of magnitude of their activation energy.

During the past 50 years, numerous theories⁶ have been proposed to explain thermally induced injuries in living organisms. Before applying the above criteria to the mechanisms involved in these theories, it is necessary briefly to characterize the attributes of a living cell.¹²

The living cell appears to consist of a semirigid relatively nonsoluble framework (e.g., nucleus, nuclear wall, and cell wall) that is primarily protein in nature. This aggregate is bathed in an aqueous intracellular fluid which contains both particulate (e.g., micellar) and soluble constituents ranging from simple ions to proteins of extraordinary complexity. Aside from certain purely physical attributes (e.g., permeability, contractibility, elasticity, cohesiveness, rigidity, and tensile strength), this protoplasmic entity respire, excretes, synthesizes all imaginable types of molecules, utilizes and liberates energy, and reproduces in a manner that perpetuates its own kind. This exceedingly complex metabolic activity is apparently both catalyzed and precisely controlled by a multiplicity of enzymatic proteins and functionally allied molecules which contribute to both the structural framework and the cytoplasmic fluid.

In view of this complex picture, no theory of thermal injury can be considered tenable unless it takes into account these completely integrated and precisely balanced phenomena, which, taken as a whole, comprise cell life. Unfortunately our knowledge of these phenomena is as yet meager and is limited to isolated observations on living protoplasm (e.g., cell respiration, mitosis, diffusion of a few substances through cell walls) and to certain chemical and physical properties and functions of a few of the molecules that can be extracted in a presumably unaltered state from dead cell brei.

Nevertheless, even on the basis of this limited information, it is interesting to speculate with regard to the general kinds of mechanisms that may be of importance in explaining the quantitative time-temperature relationship that results in irreversible epidermal injury as judged morphologically. These injury data (Sections 17.6.5 and 17.9.3) showed that episodes of transepidermal injury are quantitatively predictable by a rate equation with an activation energy of 150 kcal/mole over the entire experimental skin temperature range (44 to 70 C).

The theories⁶ that have been advanced to explain

thermal injury may be classified into three general groups.

1. *Thermal alterations in proteins.* In view of the many varied functions of proteins in the maintenance of normal cell life, it is obvious that even minor thermally induced alterations of these molecules may result in profound irreversible injuries. Thus, for example, these thermal protein changes could produce an increased permeability of the nuclear and/or cell wall, structural alterations in the nucleus itself, disintegration of the protein mitochondria present in the cytoplasm, inactivation of enzymes.

Many quantitative studies^{9,20,44} have been made of the effects of temperature on proteins, and alterations that proceed at a measurable rate between 0 to 100 C with activation energies in excess of 50 kcal/mole are not unusual. The heat inactivation of invertase ($\Delta E = 110$ kcal at pH 4 and $\Delta E = 52$ kcal at pH 5.7) and of peroxidase ($\Delta E = 189$ kcal), and the heat denaturation of egg albumin ($\Delta E = 132$ kcal at pH 5) and of hemoglobin ($\Delta E = 76$ kcal at pH 6.8) are a few of the many examples.

Thus, the morphological observations of protein dissolution and/or coagulation on which the quantitative judgment of transepidermal necrosis is based may well be directly due to the thermal alterations of as yet unknown proteins present in epidermal cells.

2. *Other possible alterations in metabolic processes.* Since temperature affects, to a greater or lesser degree, the kinetics and thermodynamics of all chemical and physical phenomena, heat may cause alterations in metabolism irrespective of its effect on proteins. For example, the entire metabolic equilibrium may be upset because of concentration changes in some of the individual constituents as a result of temperature variations both in rate of diffusion and formation and degradation of the chemical reactants comprising the process; in fact, because of this abnormal functioning, certain metabolites normally present may completely disappear and/or others abnormal and toxic in character may arise. There can be no doubt that these phenomena do take place and that they may cause cell death.

Many of these metabolic reactions,^{9,44} both enzyme- and nonenzyme-catalyzed, have been studied as *in vitro* processes, and activation energies usually between 10 and 20 kcal/mole are found. In certain instances the activation energies are less than 10 kcal/mole but none have been found to exceed 50 kcal.

Thus, to date, there is no experimental evidence

that these types of reactions can lead to a temperature coefficient for thermal injury which corresponds to that found experimentally for transepidermal necrosis.

3. *Nonprotein-induced alterations in the physical characteristics of cells.* In this group are placed all physical phenomena that are characteristic of protoplasm but are not primarily effected by the thermal alterations of proteins contained therein. For example, diffusion of metabolites through a cell wall that has not undergone chemical alteration is a member of this class, whereas changes in diffusion rates that are the result of an increased cell wall permeability due to the degradation of structural protein are specifically excluded, since this phenomenon is classified under group (1).

All of the biophysical rate processes that have been studied, such as diffusion through liquids and membranes, viscosity, rigidity, tensile strength, liquefaction, possess activation energies that are usually less than 5 kcal/mole, and never in excess of 15 kcal/mole.

Although these types of mechanisms are undoubtedly potentially capable of causing cell death, they are not the instigators of the morphological changes that are observed in irreversible epidermal injury.

Since many fatlike substances are known to melt around 45 C, the liquefaction of lipoids has received considerable consideration as a potential instigator of thermal injury.⁶ From a kinetic viewpoint, the rate of melting is a physical process with essentially a zero activation energy. This theory would predict a sharp temperature threshold for injury, with the injury rate becoming nearly a linear function of the increment in temperature above threshold value. Hence, although liquefaction might account for the quantitative epidermal thermal relationships at skin surface temperatures between 45 C and 48 C, there would be extreme variance with the experimental data at the higher skin temperatures. The extent to which thermal liquefaction of lipoid substances may contribute to cell death in tissues other than the epidermis was not investigated.

In view of the preceding discussion, it can be concluded that the only biokinetic phenomena known to date that can account for epidermal cell death are the thermally induced changes in protein structure which have an activation energy in the neighborhood of 150 kcal/mole. This in no way excludes the injury propensity of the innumerable mechanisms implied above, but merely states that all quantitative

studies made in this investigation indicate that the morphological changes (see Section 17.7.3 and Section 17.7.4) observed in the epidermal tissue can be ascribed to these protein alterations.

As to the number and kinds of proteins involved, the specific nature of thermally induced reactions,^g and the individual rate of each protein alteration, nothing can be stated. Further, it is probable that at any given hypothermic level any one of these numerous protein alterations is potentially capable of producing cell death.

17.8.3 Thermal Injury and Entropy and Free Energy of Activation

With no intention whatsoever of implying that the thermal effects on living protoplasm can be ascribed to the alteration of any single protein, it is of value to make for the moment this extreme oversimplification in order to interpret the significance of the numerical value of A in the empirical rate equation (7) which predicts completely the thresholds of transepidermal necrosis.

In vitro studies on both enzymatic and nonenzymatic proteins have shown that the rate of thermally induced changes is first-order and the quantity of degraded protein is given by²⁰

$$\ln\left(\frac{P}{Pa}\right) = \frac{K(T + 273)}{h} (e^{\Delta S/R}) (e^{-\Delta E/R(T + 273)})t. \quad (17)$$

P is the amount of protein originally present. Pa is the amount of unaltered protein present at the time t in seconds. K (1.37×10^{-16} erg/degree), h (6.55×10^{-27} erg second), and R (2 cal/degree/mole) are the Boltzmann, Planck, and gas constants, respectively. T is the temperature in C. ΔS and ΔE are the entropy

and activation energy of the rate process, respectively.

Comparing this equation with equation (9) where $A = 3.1 \times 10^{98}$ and $\Delta E = 150,000$ kcal/mole remembering that $\Omega \equiv 1$ for the production of transepidermal necrosis, we find, in the neighborhood of 50 C, the following numerical relationship for ΔS in cal/degree/mole.

$$\Delta S = 398 + 2 \ln \left(\ln \frac{P}{Pa} \right) \quad (18)$$

where Pa/P is that fraction of the original protein present which must be thermally altered in order to produce epidermal necrosis.

This equation is extraordinarily insensitive to the ratio of P/Pa . Thus, the variation of this ratio from 100 (99 per cent protein alteration requisite for injury) to 1.01 (1 per cent alteration required), changes ΔS from 401 to 389. In view of this fact, this analysis can be at once generalized to include all of the simultaneous inactivations and denaturations of the numerous protoplasmic proteins which are thermally induced and proceed with an activation energy of 150 kcal/mole.

Thus, for the combined effect of these processes, it is found that

$$\Delta S \approx 395 \text{ entropy units.} \quad (19)$$

The free energy of activation ΔF can be computed from this entropy of activation and the experimental activation energy [150 kcal/mole, equation (10)] from the following thermodynamic equation.²⁰

$$\Delta F = \Delta E + T\Delta S. \quad (20)$$

And in the neighborhood of 50 C (323 A), the following is obtained:

$$\Delta F \approx 22 \text{ kcal/mole.} \quad (21)$$

The occurrence of high energies of activation together with large increases in entropy which lead to free energies of activation from 20 to 30 kcal in the neighborhood of 50 C is a unique characteristic of all rates of denaturation of proteins and inactivation of enzymes that have been quantitatively studied *in vitro*.²⁰

Certainly, the complete quantitative concordance of the numerical constants of an experimental equation that predicts epidermal necrosis with the known effects of heat on *in vitro* alterations in protein structure is more than coincidence. Considerable confidence can thus be placed in the statement that thermally induced injury to living epidermal protoplasm is primarily due to changes in some of the nuclear and

^g Crozier and his co-workers^{9,24} have used the concept of activation energy to interpret many life processes by means of some master reaction. This interpretation has been criticized by numerous investigators,^{9,24} since it is mathematically demonstrable that the constancy or inconstancy of the activation energy is neither a necessary nor sufficient condition to prove the respective existence or nonexistence of a specific master reaction. In Crozier's investigations, the activation energies found were usually in the neighborhood of 10–20 kcal and, since the great majority of biological reactions are in this range, these criticisms are well justified. However, it is also mathematically demonstrable that no conceivable combinations of a series of reactions with activation energies within a certain bound can produce an overall kinetic process with an activation energy out of this bound (e.g., no combination of reactions for which $10 \text{ kcal} > \Delta E < 20 \text{ kcal}$ can lead to any overall phenomena with an activation energy less than 10 kcal or greater than 20 kcal). Thus, the interpretation in the text is valid.

cytoplasmic proteins which have activation energies for thermal degradation in the neighborhood of 150,000 cal/mole.

17.8.4 Latent Thermal Injury

In Section 17.6.7, the existence of latent or morphologically unrecognizable epidermal cellular injury after certain apparently harmless thermal exposures was proved by the repeated applications of subthreshold exposures. Furthermore, the time required for recovery from these latent exposures became longer the nearer they approached the threshold of microscopic visibility.

The concept of an unknown but definite fraction of certain of the cellular proteins that must be thermally altered in order to result in morphologically recognizable injury is in accord with these experimental data.

During a heat exposure that results in latent injury, a noncritical fraction of these proteins is altered. At the termination of the heat exposure, the epidermis rapidly approaches normal temperature (Section 17.3.2) and at least partial cell function is resumed. Thus, during the recovery period, the thermally altered proteins are replenished to a degree which depends, in part, upon the length of the recovery period, and, in part, upon the duration of the heat exposure which produced unrecognizable injury.

17.8.5 Summary

The numerical constants of an experimental equation, which quantitatively predicts the morphological episodes incident to transepidermal necrosis, have been subjected to theoretical analyses. It is demonstrated that, of all of the known biokinetic phenomena, only thermal alterations in cellular proteins that have an energy and entropy of activation of 150 kcal/mole and 395 entropy units, respectively, will account for the experimental observations. This theory is also in agreement with the latent thermal injury data given in Section 17.6.7.

17.9 EXPOSURE TO HOT AIR AND RADIANT HEAT

17.9.1 Introduction

In preceding sections of this chapter it has been shown that a very brief exposure of an animal to excessive circumambient heat may cause rapid circulatory collapse and death. It was found that transfer of heat to and through the skin was a more important

cause of such casualties than was the effect of heat on the respiratory tract. In a quantitative and pathological study of the effects of hot water on the skin it was shown that certain predictable and reproducible reciprocal relationships exist between the intensity and duration of an episode of hyperthermia and its capacity to destroy the epidermis.

These findings suggest that similarly reproducible and predictable relationships may exist between the intensity and duration of an episode of hyperthermia and casualty production by exposures to hot air and radiant heat such as may occur incident to a conflagration or to a flame thrower attack.

To determine whether or not such is the case a series of experiments was undertaken in which pigs received generalized cutaneous exposures for varying periods of time to circumambient (and circumradiant) temperatures that varied between 70 and 550 C. The cutaneous and systemic effects of these exposures on animals were correlated with exposure time and source temperature.

17.9.2 Experimental Procedure

Previously clipped and anesthetized pigs were fastened on a platform in the manner shown in Figure 29 and a preheated oven was lowered over them. In most of the experiments the snout of the animal protruded through an aperture in the bottom of the platform. There were two advantages in this arrangement, one being protection of the respiratory tract and the other being that it was possible thereby to determine the time of death of animals that succumbed during the period of exposure.

The source of heat was a bottomless oven constructed of iron and firebrick and having a capacity of approximately 1,100 l. The box weighed 2,700 kg and its internal measurements were 89x91x130 cm. Chromel alumel (10 gauge) thermocouples welded onto the inside plate of the box provided information as to the source temperature during the period of pre-exposure heating as well as during the period that the animal was being exposed. To heat the box, it was lowered into a vertical gun annealing furnace^b (Watertown Arsenal). When it had become thoroughly heat-soaked and was at a slightly higher temperature than that at which it was desired to expose the animal, the oven was quickly withdrawn from the furnace by an overhead crane and lowered over the platform on which the animal was sus-

^b These facilities at the Watertown Arsenal were made available through the courtesy of the War Department.

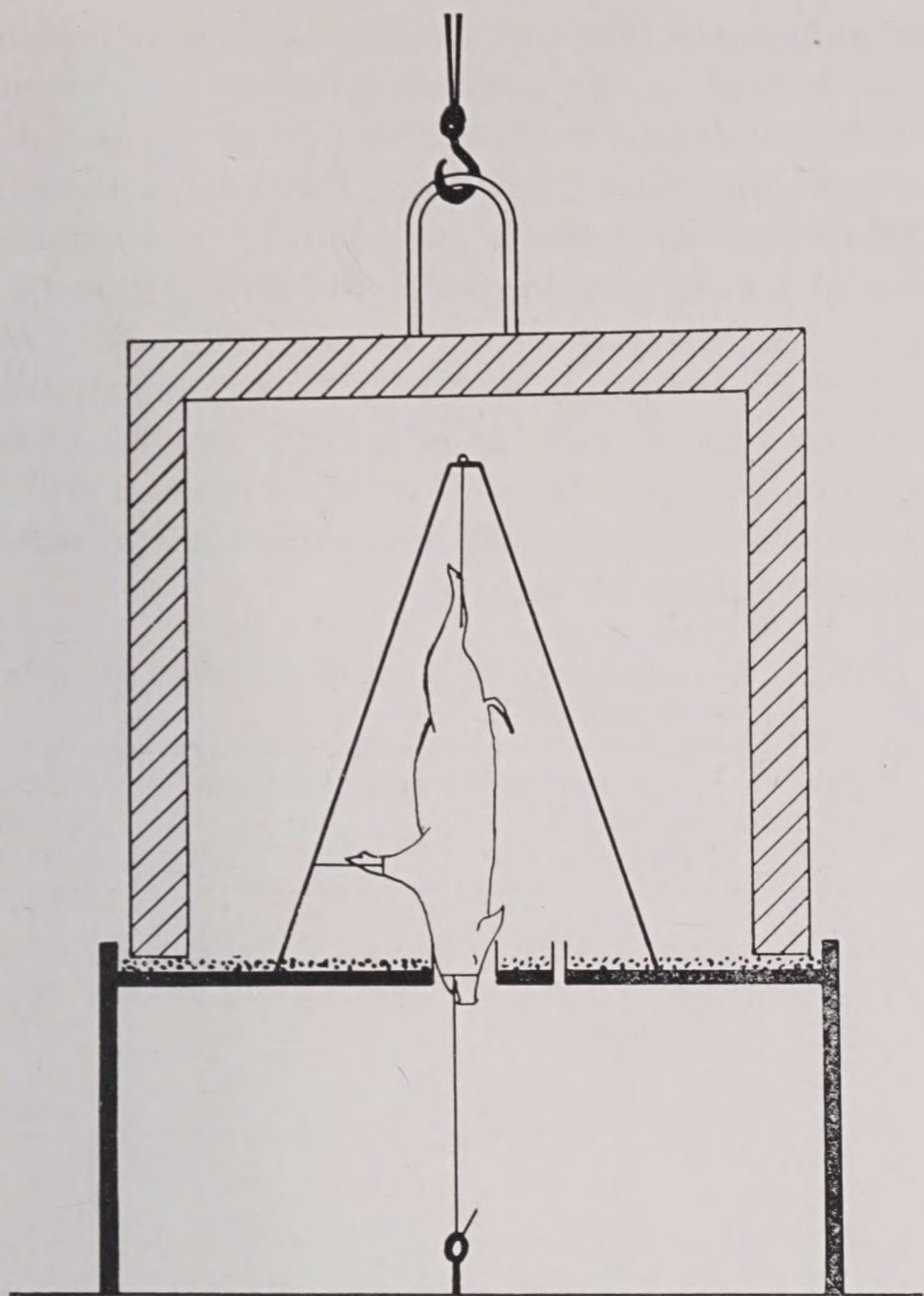


FIGURE 29. Method of exposing animals to hot air and radiant heat at Watertown Arsenal. Heavy iron and firebrick box was preheated in gun annealing furnace and lowered over platform.

pendent. The interval required for the descent of the box from the top of the tripod to the floor of the platform was between 3 and 4 seconds.

The platform supporting the tripod upon which the animal was suspended was elevated 75 cm above the floor and covered by a layer of dry sand. In addition to the aperture to accommodate the snout of the animal, there were other openings in the platform through which wires could be passed to the temperature recording equipment.

Three 28 gauge iron-constantan thermocouples connected in parallel were fastened to the surface of the animal in such a way that the junctions were separated from the skin by a distance of between 2 and 5 cm. These provided for a continuous recording of ambient temperature.

Rectal temperatures were taken routinely. In some experiments a rectal thermocouple provided for a continuous record. In others the temperature was taken by thermometer before and at intervals after exposure. On several occasions the postexposure

temperature of the right auricular blood was taken for comparison with that of the rectum.

In a number of experiments a 28 gauge iron-constantan thermocouple contained in a venipuncture needle was inserted into the dermis to record the temperature of the subepithelial connective tissue during and after exposure.

Temperature of air in different parts of exposure chamber: Values given in the text for ambient temperature refer to the mean temperature of the air in which the animal was enveloped. The thermocouples by which the ambient temperature was measured were routinely placed in approximately the same positions in relation to the animal in all experiments. One was fastened to the skin just below the base of the tail and one on each side of the mid-portion of the body. It was regularly observed that the mean ambient temperature was approximately 20 per cent lower than that measured by the thermocouples incorporated in the wall of the exposure chamber. Although the rate of cooling of the exposure chamber (and the air contained by it) varied according to the magnitude of the initial difference between its temperature and that of the room, the drop was never in excess of 5 per cent in experiments lasting 15 minutes or less.

Because of the convection currents that resulted from the difference between the temperature of the surface of the animal and that of the air surrounding it, the temperatures recorded in various parts of the exposure chamber showed remarkably little variation. Thus in the mid-horizontal axis of the chamber difference in temperature was less than 5 per cent from a point 15 cm internal to the wall to a point 15 cm external to the animal. In the mid-vertical axis there was less than 15 per cent difference in the temperature of the air between a point 15 cm below the roof and a point 15 cm above the floor of the exposure chamber.

MEASUREMENT OF HEAT TRANSFER

Under these conditions, there were three mechanisms by which heat could be transferred from the hot walls of the box to the surface of the animal, namely, air conduction, air convection, and infrared radiation. The energy transferred by conduction and convection is hereafter designated as ambient, and that transferred by radiation as radiant. Although the relative importance of these two types of heat transfer can be directly computed by means of equations (1) and (2) of Section 17.3, it was decided to verify

these calculations under the conditions that prevailed in these experiments. Unfortunately, direct determinations of the ambient and radiant caloric uptakes of animals were not feasible and it was necessary to measure these values by means of calorimeters suspended in the center of the exposure chamber preliminary to animal experimentation.

The calorimeters consisted of copper cylinders which measured 2.5 cm in diameter and 5 cm in length. One of each pair of cylinders was gold-plated and the other blackened with colloidal graphite (aquadag). Thus, the former measured only ambient energy, whereas the latter determined both ambient and radiant energy.

The caloric uptake rate of the calorimeters was readily calculated from their known heat capacity and surface area and the experimentally determined rate of temperature rise as measured by an iron-constantan thermocouple soldered within the calorimeter. Because of the discrepancy between the size of these calorimeters and that of the pigs (approximately 30x75 cm), it was necessary to multiply the ambient calorimetric measurements by a numerical factor equal to 0.5. Since the skin is known to be a nearly perfect black body for the radiation emitted under these experimental conditions and since the dimensions of the exposure chamber were large with respect to those of the animal, the radiant caloric measurements are directly applicable.

Actually, these data, so corrected, apply to a metallic cylinder of dimensions similar to those of a pig. Since it has been shown that under the conditions of experimentation these data would be equally applicable to both smooth and rough and to metallic and nonmetallic surfaces, it is believed that they represent a true estimation of the caloric uptake rate of pig skin.

The data given in Table 18 are an estimation of the radiant and ambient caloric uptake rate per square centimeter per minute of pig skin when the surface temperature is 35 C. It is obvious that during the heat exposure the surface temperature increases with time, resulting thereby in a corresponding decrease in the rate of caloric uptake. For skin surface temperatures not greater than 60 C, caloric uptake rates are directly proportional to the difference between the temperature of the surrounding air and that of the surface of the animal. Thus, for surface temperatures below 60 C the requisite caloric uptake rates can be computed from these data. Further examination of Table 18 shows that the infrared radi-

ation from the inside walls of the box was the principal source of heat energy absorbed by the animals. Under conditions that produced an air temperature of 70 C, this contribution was 50 per cent, whereas at 500 C it was 85 per cent. These percentages remained nearly invariant throughout the entire time of a given heat exposure. As previously indicated, these values for the nonradiant and radiant contribution to caloric uptake rate can be directly computed from equations (1) and (2) and, if this is done, it will be found that they agree with the experimental values to within about 15 per cent.

TABLE 18. Estimated caloric uptake for pig when skin surface temperature is 35 C.

Air temperature C	Caloric uptake in cal/cm ² /min			Per cent of total radiant
	Nonradiant (ambient)	Radiant*	Total	
70	0.2	0.2	0.4	50
100	0.5	0.6	1.1	55
150	1.0	1.4	2.4	58
200	1.7	2.6	4.3	61
250	2.2	4.2	6.4	65
300	3.0	6.2	9.2	68
350	3.8	9.8	13.6	72
400	4.5	17.0	21.5	79
450	5.5	24.0	29.5	81
500	6.5	35.0	41.5	85

* Because of the difference between the air and source temperature when animals are placed in the exposure chamber, these radiant data refer to a source temperature 20 per cent in excess of the tabulated ambient temperature.

17.9.3 Effects on Animals

The results of 71 individual exposures of pigs are shown in Figure 30. It was at first intended to present in this chart only the data derived from 49 experiments in which pigs of uniform weight (7 to 18 kg) received generalized (approximately 90 per cent) cutaneous exposures to heat. The additional 22 experiments included those in which large animals (in excess of 15 kg) were used, those in which hot air was breathed during the time that the skin was being exposed, and those in which the animals were anesthetized after rather than before exposure. When it was found that there were no significant differences in the experimental results that could be related to the body weight of the animals (7 and 32 kg) or to anesthesia it was decided to present all experimental data in one chart.

The temperature and duration of each exposure is indicated by the position of the individual experiments on the grid. The vertical points of reference on the left are in logarithmic progression and represent the internal temperature of the exposure chamber,

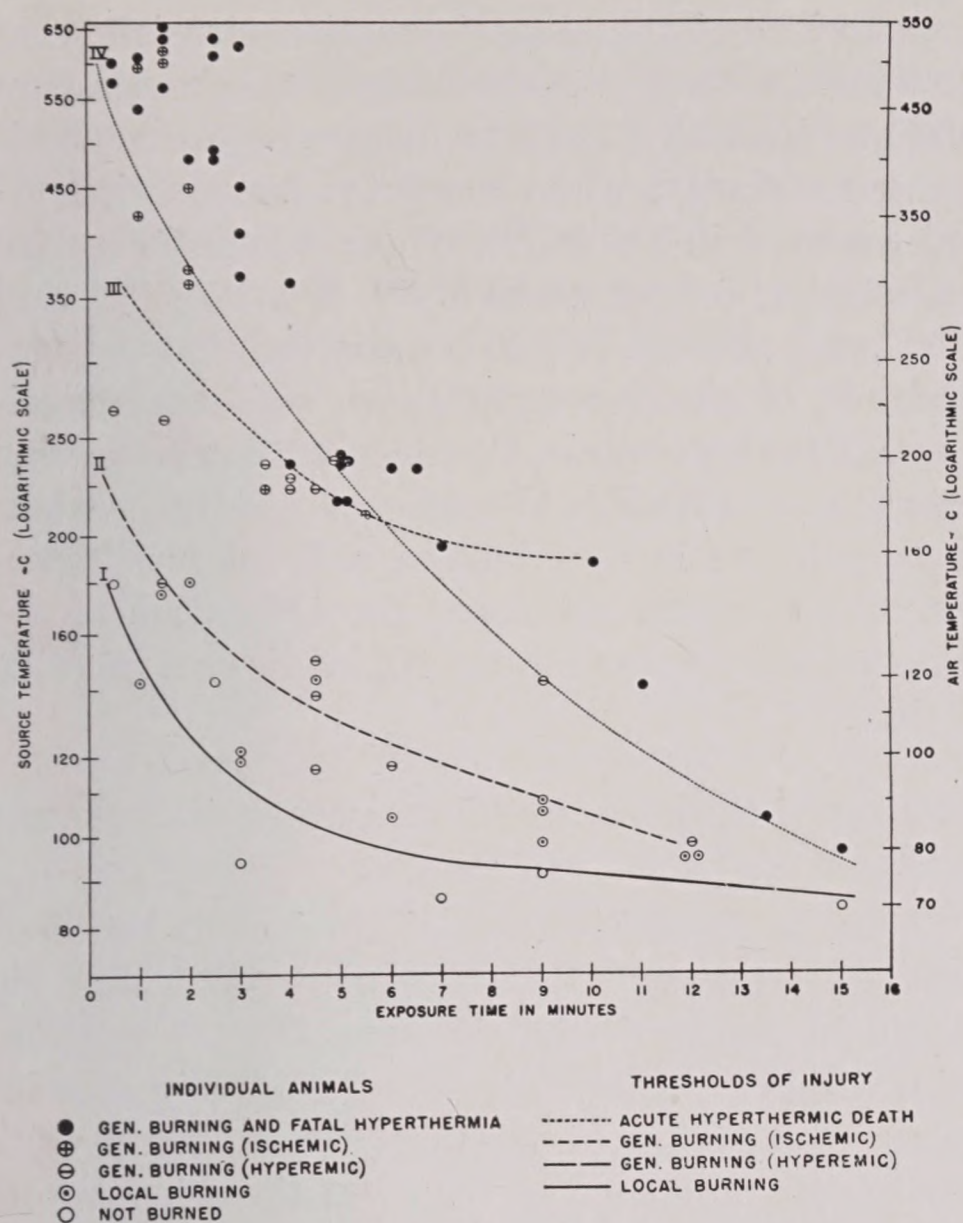


FIGURE 30. Graph showing results of 71 experiments in which pigs received generalized cutaneous exposures to ambient and radiant heat in oven. Each experiment is depicted by circle. Duration and temperature of exposure are indicated by position of circle in grid. Effect of exposure on pig is shown by character of circle. Curved lines traversing grid depict approximate thresholds at which varying degrees of cutaneous and systemic injury occurred.

whereas those on the right represent the corresponding ambient temperature in the vicinity (within 5 cm) of the animal. The horizontal points of reference indicate the duration of the exposure period.

It may be seen that the experiments fall into three main groups with respect to their effects on the pigs. Some animals were unharmed and developed neither superficial nor systemic evidence of injury, others received cutaneous injury with insufficient systemic disturbance to result in early collapse and death, and still others died during the exposure or within 30 minutes after the exposure.

The upper limits of exposures which pigs survived without either cutaneous injury or severe physiological disturbance are indicated by the line (I) that traverses the grid from left to right. Exposures lying below this line failed to cause cutaneous burning. Exposures lying between the first and second lines characteristically resulted in mild or localized burn-

ing. The second line (II) represents the approximate threshold at which generalized second-degree burning occurred. The third line (III) represents the approximate threshold at which the burned skin and subcutaneous tissue underwent ischemic coagulation. The skin of most pigs that received exposures lying above this threshold was pale and the loss of elasticity of the coagulated superficial tissues resulted in the formation of deep fissures when the extremities were flexed. The uppermost line (IV) represents the approximate threshold at which rapidly fatal systemic hyperthermia occurred. Most pigs receiving exposures in excess of this threshold died within a few minutes (usually under 15 and occasionally as long as 30) after the oven had been lifted from the platform.

Comparison of effects of hot air and hot water exposures: Injury by heat is determined by the degree and duration of the rise in tissue temperature. It will be shown that for the same kind of skin the production of a given degree of thermal injury depends only on the time-temperature relationships within the tissue irrespective of the source of the heat. Since threshold II in the hot air experiments (see Figure 30) depicts the occurrence of transepidermal necrosis, it can be inferred that for the same source temperature actual tissue temperatures attained were considerably lower than those in hot water experiments (see Figure 14).

In Figure 31 are depicted the source temperature-time relationships that were required to produce transepidermal necrosis in both the air and water exposures, where in the latter case the surface of the skin was maintained at essentially the same temperature as that of the source. A comparison of the two curves shows that a 15-minute exposure to water at 48 C was sufficient to produce approximately the same degree of injury as that which resulted from a 15-minute circumambient and radiant exposure at 75 C. A hot water exposure for 1 minute at 53 C produced about the same degree of injury as resulted from a 1-minute exposure at 160 C to ambient and radiant heat. It is apparent, therefore, that the actual surface temperatures responsible for the kind of irreversible injury observed at threshold II in Figure 30 were considerably lower than the recorded ambient temperatures at which they were produced. In the hot water exposures the change in tissue temperature with time was determined by the rate of heat flow through the skin, whereas in the oven exposures it was limited by the rate of heat transfer to the surface.

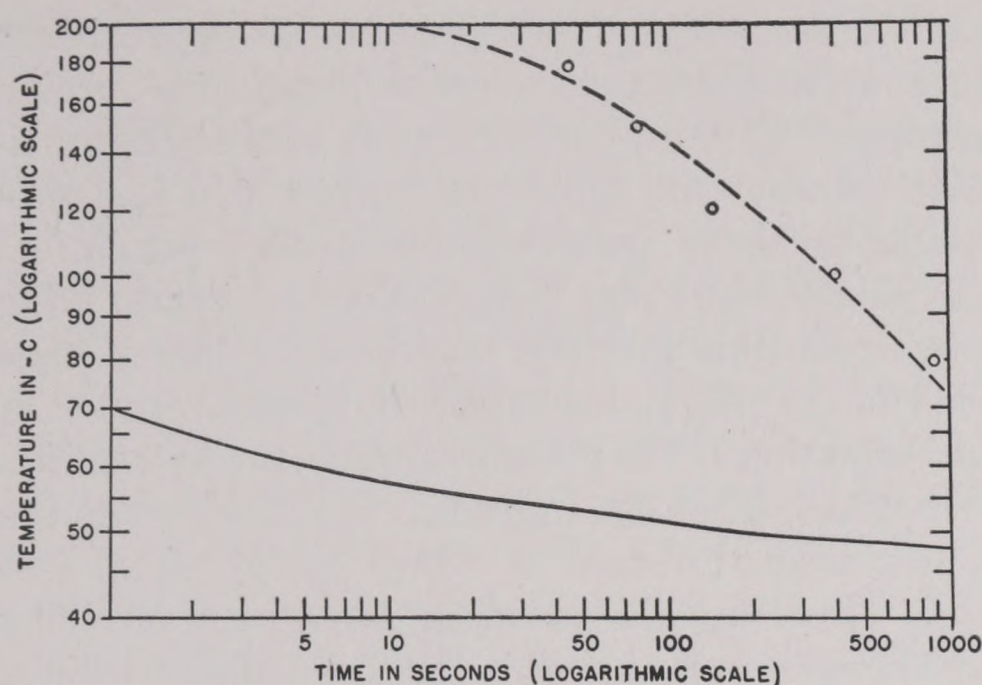


FIGURE 31. Solid curve depicts time-source temperature relationships requisite to complete transepidermal necrosis when skin site is exposed to flowing water of constant temperature. Dotted curve shows time-air temperature relationships (curve II of Figure 30) that produce similar degree of injury when skin surface is surrounded by an envelope of radiant and ambient heat (oven experiments). Open circles show values which were computed by means of equations (1), (2), (Section 17.3) and (15) (Section 17.7).

The actual time-temperature relationships within the epidermis under these experimental conditions have been computed by equation (6), which results from the application of the general theory of heat to this problem (Section 17.3.1), and are reported in Table 7B (Section 17.3.2). These data show the rate of increase in the epidermal temperature incident to an exposure to an envelope of radiant and ambient heat. It is apparent in the case of a generalized exposure that long before the temperature of the surface of the skin would approach that of the air, the animal would have succumbed to a generalized hyperthermia.

In Section 17.6.5, the degree of epidermal destruction was shown to be mathematically predictable by means of equation (15), so long as T_t , the time dependence of the basal epidermal temperature, is known. This equation was developed empirically from data pertaining to the degree of epidermal injury when the skin surface was immediately brought to and maintained at a constant temperature (hot water experiments). It was stated that equation (15) should predict the time required to produce all thermally induced transepidermal injuries which result from any conceivable type of heat application, so long as the time dependence of the temperature at the dermal-epidermal junction during the heat exposure is known.

The five circles depicted in Figure 31 are the result

of numerically integrating¹⁷ equation (15) following the substitution of the epidermal time-temperature relationships which result from an exposure to ambient and radiant heat as computed by equation (6) and recorded in Table 7B. These calculations were made for air temperatures of 80, 100, 125, 150, and 175 C, respectively. It is to be observed that the concordance of these computations with the experimental data is excellent. Considerable confidence can thus be placed both in the statement of the previous paragraph and in the "infinite body picture" (Section 17.3.1) which permitted the estimation of the temperatures attained in the epidermis as a function of time.

PROBABLE EFFECTS OF COMPARABLE EXPOSURES ON MAN

So far as the skin effects of ambient and radiant heat are concerned, the reactions in man and pig should be similar if the time-temperature relationships within the epidermis were the same in each instance (see Figure 14, Section 17.6.4). However, a predictable difference in these relationships during identical heat exposures of this type arises from the fact that sweating of human skin can undoubtedly increase the time threshold at which cutaneous burning occurs.

That sweating can afford considerable protection in the case of relatively low-intensity hot air exposures can be assumed from the fact that man may lose moisture by this mechanism at the rate of approximately a liter per hour. This could result in heat loss at the rate of between 0.5 to 1.0 cal/min/cm² of skin surface. Heat loss by porcine skin through vaporization of moisture is relatively slight (approximately 0.1 cal/cm²/min). See Section 17.5. Thus, in view of the caloric uptake data presented in Table 18, it is possible that the time threshold for cutaneous burning in man is appreciably longer than that for the pig for all circumambient and radiant temperatures lower than about 120 C. That such a degree of protection would be afforded at higher air temperatures is unlikely since it would be necessary to assume that sweating was already established at a significant level at the moment of exposure and that all of the sweat excreted was vaporized. No experiments were conducted to establish the quantitative extent to which sweating may be capable of protecting human skin against thermal injury to either low or high ambient and/or radiant temperatures.

It should be emphasized that these data refer only

to unclothed animals. It is possible to estimate the degree of protection afforded by clothing by a knowledge of their impedance to the heat reaching the skin surface, but since this thermal impedance is so dependent upon the physical characteristics of the fabrics involved, upon tightness of fit, and upon the type of heat exposure, further consideration of this problem is not warranted in this chapter. The method of obtaining these thermal protectivities of clothing under specific experimental conditions is given in detail elsewhere.^{4a}

DEATH OF PIGSⁱ

It may be seen from Figure 30 that rapidly fatal physiological disturbances resulted from a wide range of thermal exposures and that at any given temperature within the range investigated survival or death was determined by the duration of the exposure period. Observations were made on the various pathological and physiological changes resulting from sublethal and lethal cutaneous exposures to heat.

PATHOLOGICAL CHANGES

There was no apparent relationship between the occurrence of early death and the severity of the cutaneous injury. Some animals that died during or soon after exposure at relatively low temperatures showed remarkably little evidence of cutaneous injury. Others that received extensive third-degree burns at higher temperatures survived many hours after exposure and showed no systemic evidence of impending death at the time they were sacrificed. It was obvious that the cutaneous lesion *per se* was not responsible for early collapse and death.

Apart from cutaneous burning there were no significant differences in the pathological changes observed in animals that died following short exposures at high temperatures and in those that died following longer exposures at lower temperatures. The most constant post-mortem finding in all animals that died within 30 minutes after exposure to heat was the presence of widely disseminated small and large focuses of hemorrhage throughout the internal viscera. These were seen most frequently and prominently beneath the endocardium of the right and left ventricles. Another site of predilection for such hemorrhages was the gastric and duodenal mucosa.

The right auricle was characteristically dilated and

filled with dark red unclotted blood. The impression was gained that the ventricles were more frequently found in the state of contraction after high- than after low-intensity exposures.

The lungs of pigs that died during or soon after cutaneous exposures to excessive heat rarely showed more than a mild degree of pulmonary edema, in contrast to those of dogs and goats, in which systemic hyperthermia characteristically led to moderate or severe pulmonary edema.

Animals sacrificed 12 to 24 hours after severe cutaneous burns had been sustained frequently showed severe parenchymatous degeneration of adrenal cortex, liver, and renal tubular epithelium. Hemoglobin casts were sometimes observed in the collecting tubules of the kidneys and the urine of burned animals regularly contained large amounts of blood pigment.

CHANGES IN BLOOD

Examination of the blood of burned animals regularly showed intravascular hemolysis. That intravascular hemolysis was not a determining factor in survival was indicated by its absence in animals that died after low-intensity exposures. A more complete discussion of the relationship between intensity of thermal exposure and hemolysis will be found in Section 17.10.

Examination of wet and dry smears of blood of severely burned animals disclosed microspherocytosis and disintegration of erythrocytes (see Figure 32). These changes were similar to those observed in the blood of burned human subjects, by Shen, Ham, and Fleming.⁴² They were not observed in the blood of animals that died after low-intensity thermal exposures. In severely burned animals there was an increase both in the clotting time and in the fragility of erythrocytes.

Plasma Turbidity. The observation of turbidity of the plasma together with the finding in some fatally burned animals of small agglomerates of protein and enmeshed cells in wet smears of blood^{4b} led to a re-investigation of a phenomenon described by Kabat and Levine.²⁷ These observers reported that the intravenous injection into a cat of 4 ml of heated *citrated* plasma caused immediate death. After centrifugalization, they found that the supernatant fluid of such plasma produced no ill effects, whereas death resulted from the intravenous injection of the resuspended sediment.

A repetition of the experiments of Kabat and

ⁱ Several goats and dogs received exposures estimated to be lethal or sublethal for pigs and the impression was gained that their susceptibility to fatal systemic hyperthermia did not differ significantly from that of the pig.

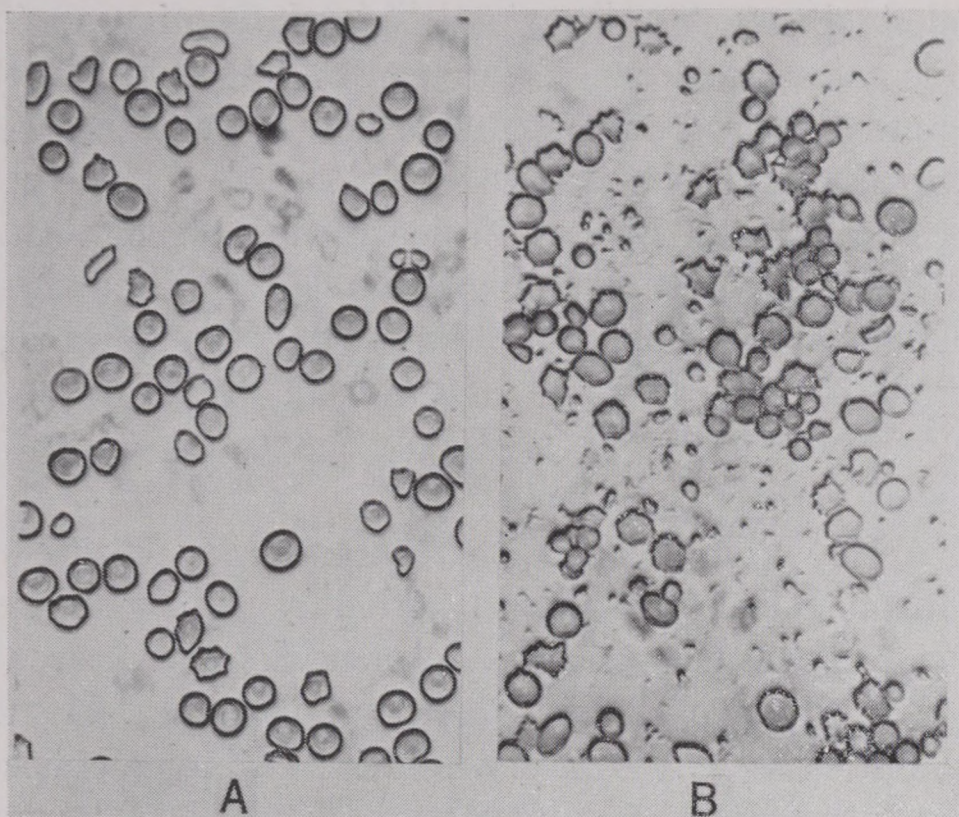


FIGURE 32. Blood smears of pig No. 856 (9.1 kg) before (A) and 3 minutes after (B) 5-minute exposure to hot air and radiant heat at ambient temperature of 180 C. Animal received third-degree burns of about 85 per cent of body surface and died 3 minutes later with rectal temperature of 43.5 C. Examination disclosed intravascular hemolysis, plasma potassium concentration of 19.4 milliequiv/l and disintegration of erythrocytes as shown in (B). During exposure, temperature at interface between dermis and subdermal fat, as recorded by needle thermocouple, rose to maximum of 63 C.

Levine resulted in the observation that blood pressure fell rapidly and that sometimes animals died following the intravenous injection of a small amount of heated *citrated* plasma. However, when heparin was used as an anticoagulant instead of citrate, animals tolerated relatively large intravenous injections of heated plasma without ill effects and without significant change in blood pressure. Slight lowering of blood pressure was observed in a few animals after injection of heated heparinized plasma or the sediment of heated plasma. No deaths occurred, however, even when amounts as great as 15 ml were used.

It was concluded that the particulate masses in preheated blood described by Kabat and Levine may be deleterious to a slight degree and in combination with sodium citrate (250 mg/10 ml of blood) may cause death if injected rapidly. It is not believed, however, that these masses contributed significantly to the hyperthermic deaths observed in these experiments.

Perfusion Experiments. A heart-lung preparation (Starling method) was perfused with the blood of a dog that had died of circulatory failure 7 minutes after being immersed in hot water at 70 C. Continuous records of the heart-lung preparation included

aortic pressure (Hg manometer), systemic minute output (total output less coronary flow), ventricular volume (Henderson cardiometer with a Kiese volume recorder), and oxygen consumption (spirometer).

The preparation had been used earlier for a study of the metabolic effects of alloxan; the heart was failing spontaneously at the time the blood from the burned animal was introduced into the perfusing system (about 3 hours after the preparation had been isolated). Fifty milliliters of the blood of the heated animal were injected into the venous return during a period of 1 minute. The minute output was about 130 ml/min at the time of the injection and the injected blood reached the heart diluted two or three times with the original blood of the preparation.

Six minutes later 100 ml of the blood of the burned animal were injected again in a period of 1 minute. This was diluted no more than once with the blood of the preparation.

Six minutes later the blood in the venous reservoir was removed and replaced with 200 ml of blood of the burned animal. Following this last addition the heart-lung preparation was being perfused almost entirely by the blood of the heated animal.

In none of the three trials was there any significant change in the pressure, the minute output, the heart rate, or the oxygen consumption. Although coronary flow was not recorded, any great increase in it such as might have been expected if the blood had contained as much as 0.5 mg of histamine would have been recognized by an increase in the discrepancy between the stroke volume as recorded by the cardiometer and the stroke volume as calculated from minute output and heart rate. Such a change was not observed.

No deleterious effect resulted from perfusing the heart-lung preparation with the blood of the burned dog. Actually there was slight evidence of a beneficial effect, such as would be expected from the addition of any fresh blood after 3 hours of perfusion.

RELATION OF SYSTEMIC HYPERTHERMIA TO SURVIVAL

There appeared to be a definite correlation between survival and the height to which the internal body temperature was raised. Most of the animals that died soon after exposure were found to have a marked elevation of rectal temperature. In the case of exposures of long duration and low intensity the rectal temperature was only slightly lower than that of the blood within the right auricle. In animals that died within a few minutes after exposures of short

duration and high intensity there was characteristically a difference of several degrees between rectal and blood temperature (see Section 17.11).

The correlation between severity of systemic hyperthermia and the occurrence of early death is shown in Figure 33. With one exception all pigs that

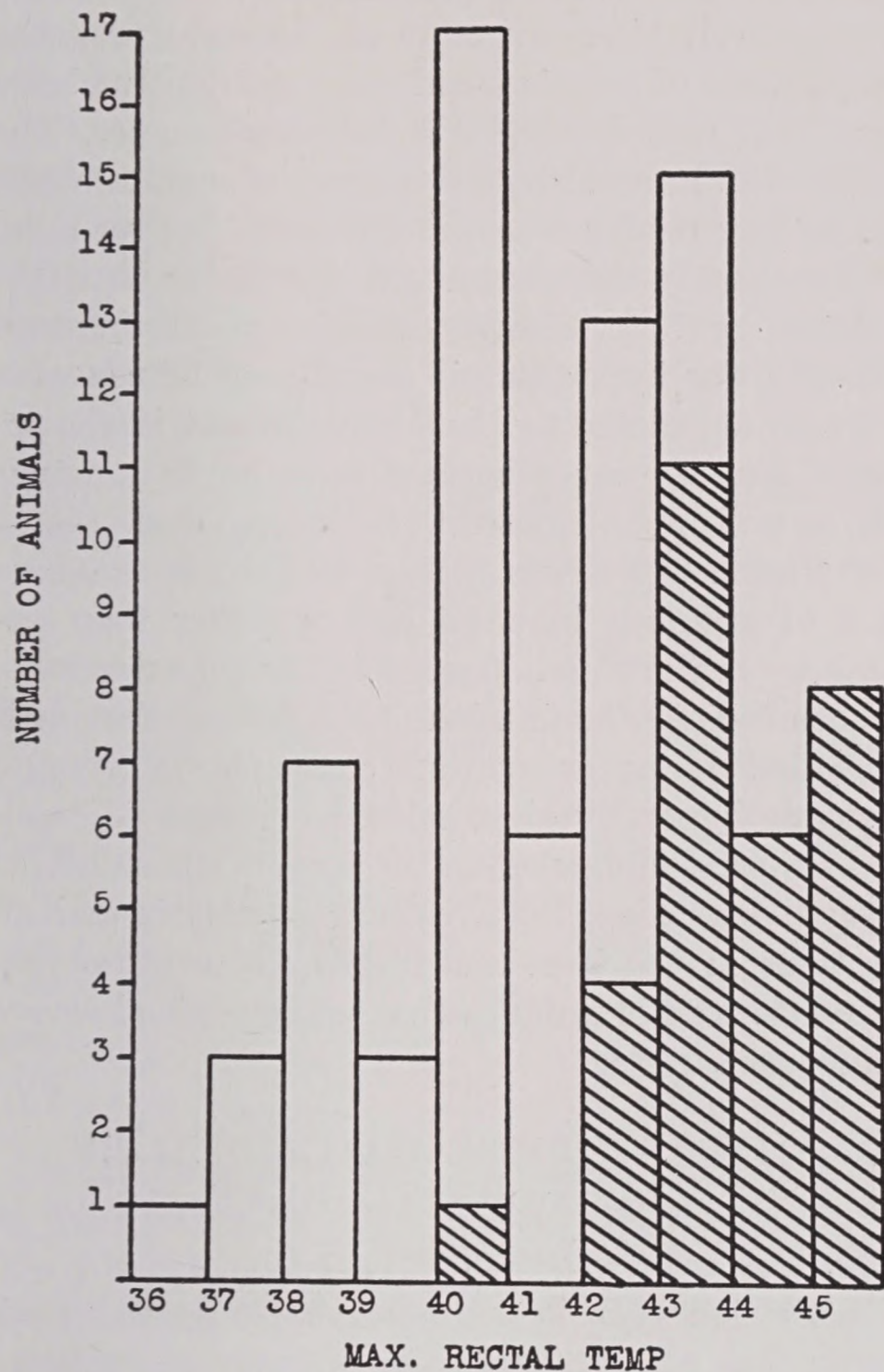


FIGURE 33. Distribution of animals according to maximum 30-minute rise in rectal temperature following exposure to hot air and radiant heat. Initial temperatures were low because of pentobarbital sodium anesthesia. Open portions of columns represent animals that survived; shaded portions, animals that died during or within 30 minutes after exposure. It is apparent that there is close correlation between systemic hyperthermia and death.

died during the early postexposure period were those that developed rectal or heart's blood temperatures of 42.5 C or higher. No pig whose rectal temperature rose to 44 C or higher survived for more than a few minutes. Eleven of the 15 that developed rectal temperatures between 43 and 44 C and 4 of the 13 with rectal temperatures between 42 and 43 C died during the episode of hyperthermia.

PATHOLOGICAL PHYSIOLOGY¹

Prior to the exposure of several pigs to hot air, insulated electrocardiographic leads were connected with the extremities and a carotid cannula was introduced. The effect of the exposure on the rate of respiration, the pulse rate, the arterial blood pressure, and the conduction system of the heart of these animals was observed.

Within a few seconds after exposure, there was a sharp increase both in blood pressure and in rate of respiration. The respiratory rate continued to increase and remained rapid for some time after the exposure was terminated. Soon after the initial rise there was a fall in blood pressure to or slightly below the pre-exposure level. In some animals, the pressure was well maintained at that level until within a few minutes before death, whereas, in others, there was a gradual and progressive decline beginning immediately at the conclusion of the initial rise. Inability to control the movements of the animal during the period of exposure made it impossible to secure satisfactory records of venous pressure.

Electrocardiographic abnormalities were observed in some animals soon after the beginning of exposure, whereas, in others, such changes did not develop until well after the onset of circulatory failure. Abnormalities observed in a few instances soon after the beginning of the exposure (within 2 or 3 minutes) included increase in rate, reduction in the voltage of the *QRS* complex, and inversion of the *T* waves. Ventricular extra-systoles were observed and as the exposure was prolonged there were greater disturbances in rhythm. Such animals developed ventricular tachycardia followed by fibrillation and death.

Although abnormalities in the electrocardiogram were sometimes observed before there was evidence of respiratory failure, the terminal and agonal fall in blood pressure usually occurred at about the same time that tachypnea gave way to intermittent periods of apnea.

Although the results of these experiments indicated that there were two types of hyperthermic circulatory failure, one central and the other peripheral, it was obvious that further and more rigidly controlled physiological experimentation was required. Such studies were not feasible in the circumstances in which the hot air experiments were conducted (see Section 17.11).

Changes in Blood Potassium. Samples of blood were withdrawn by cardiac puncture before, during, and after lethal exposures of four pigs to hot air. It was

found that the potassium concentration of pig's blood is approximately 50 milliequiv/l. The partition of potassium between erythrocytes and plasma is approximately 50 to 1.5. The postexposure plasma levels in these four animals were 7.3, 10.6, 17.4, and 19.6 milliequiv/l, respectively. The observation that cutaneous hyperthermia was capable of causing the plasma potassium to rise to 17 milliequiv/l and higher suggested acute potassium poisoning as a potential cause of death. Further investigation of the importance of potassium release to the occurrence of circulatory failure and death following exposure to heat will be discussed in Sections 17.10 and 17.11.

17.9.4 Summary

The time-temperature relationships responsible for varying degrees of cutaneous injury and for acute circulatory collapse and death incident to exposures to circumambient and circumradiant heat similar to those that may result from a conflagration or from a flame thrower attack have been determined for the pig.

At relatively low air temperatures (under 120 C) man, because of his ability to sweat, is undoubtedly less susceptible to injury than the pig. It is doubtful, however, that sweating provides a significant degree of protection at higher temperatures in which the rate of heat transfer to the skin is considerably more rapid than the rate at which it can be dissipated by vaporization of sweat.

It should be borne in mind that the relationships of source temperature to injury production derived from these experiments apply to unprotected skin and are not valid for exposures in which the skin is protected by hair or clothing.

It has been shown that the time-tissue temperature relationships responsible for transepidermal necrosis (second-degree burning) by exposure to hot water as given in equation (15) (Section 17.6) are equally applicable to exposures to circumambient and circumradiant heat.

The severity of the immediate physiological disturbances resulting from exposure to excessive heat is frequently disproportionate to the severity of cutaneous burning. Rapid circulatory collapse and death may result from exposures of such low intensity that little or no burning of the skin is sustained. Exposures of short duration at higher temperatures may cause severe and generalized cutaneous burning with remarkably little systemic physiological reaction during the early postexposure period.

The severity of the immediate physiological dis-

turbances resulting from exposure to excessive heat bears a quantitative relationship to the extent to which the body temperature is increased. Pigs in which the rectal temperature failed to rise above 42 C rarely and those in which it rose as high as 44 C invariably died of acute circulatory failure. In animals that died within a few minutes after exposure to excessively high environmental temperatures, the temperature of heart's blood was consistently higher than that recorded by a rectal thermometer. The shorter the interval between onset of exposure and death, the greater was the difference between the temperature in the rectum and that in the heart.

Although the precise physiological mechanisms responsible for hyperthermic circulatory failure were not fully elucidated by these experiments, it was apparent that the early death of some burned animals was caused or contributed to by hyperpotassemia.

Perfusion experiments failed to disclose the presence of injurious humoral agents (other than potassium) in the blood of recently burned animals.

Pathological examination of the bodies of animals that died during or soon after an episode of acute systemic hyperthermia disclosed evidence of capillary endothelial damage in the form of disseminated visceral petechiae. Intravascular hemolysis and alterations in the form and fragility of erythrocytes were observed in animals that had sustained severe cutaneous burning.

17.10 HYPERPOTASSEMIA CAUSED BY EXPOSURE TO HEAT

17.10.1 Introduction

In Section 17.9, it was observed in some experiments that cutaneous exposure of pigs to excessive heat resulted in rapidly fatal circulatory failure that was associated with marked electrocardiographic abnormalities and a sharp rise in plasma potassium to levels ordinarily considered incompatible with life.

The implication of these observations was such as to warrant further study of the effect of cutaneous hyperthermia on the concentration of potassium in the blood.

17.10.2 Experimental Procedure

Samples of blood for chemical analysis were obtained from the heart by means of an inlying jugular cannula.

Potassium determinations were carried out on the trichloroacetic acid filtrate of plasma and lysed blood according to the method of Lowry and Hast-

ings³² as modified by Cohn and Tibbetts. Hematocrit was determined in Wintrobe tubes after centrifuging for 30 minutes at 2500 rpm. The method of Bing,³³ et al, as modified by Ham,³⁴ was used for determining plasma hemoglobin. Whole blood hemoglobin was determined on 0.1 ml of 1/5 dilution of blood in 5 ml of dilute ammonia by the Klett-Sumerson colorimeter.

17.10.3 Animal Experiments²

Before undertaking further investigation of the relationship of hyperthermia to the development of hyperpotassemia, an experiment was undertaken to determine the effect of systemic anoxia on the potassium concentration of the plasma independently of hyperthermia (Table 19).

TABLE 19. Changes in the blood of a pig during and after death by strangulation.

Blood withdrawn	Volume packed cells	Hemoglobin in cells g/100 ml	Hemoglobin in plasma % hemolysis	Potassium in red cells milliequiv/l	Potassium in plasma milliequiv/l
Control	45	33	0	132	5.2
0 min		<i>Trachea clamped</i>			
4 min	49	31	0	128	9.1
8 min	48	34	0	130	9.3
8 min		<i>Animal died</i>			
68 min	?	?	0	?	16.8

A control sample of blood was taken from an 8.2-kg pig. The trachea was then exposed and clamped and after 4 and 8 minutes additional samples of blood were obtained. The animal died at the end of 8 minutes and was allowed to remain on the operating table at room temperature for an hour thereafter, at which time, the fourth and last sample of blood was withdrawn. The analytical results are shown in Table 19.

It may be seen that the plasma potassium level was almost doubled during the 8 minutes that elapsed between the onset of asphyxia and death. Most of the increase occurred during the first 4 minutes of this period. There are two obvious sources from which the increment may have been derived, one being the erythrocytes and the other the extravascular tissue. A comparison of hematocrit and hemoglobin content of cells at the end of the 4-minute period indicates that swelling of erythrocytes had occurred. The hematocrit rose from 45 to 49, whereas the hemoglobin dropped from 33 to 31 g per 100 ml of cells. It

appears that the observed decrease in the concentration of intracellular potassium from 132 to 128 milliequiv/l was probably due to swelling of red cells rather than to loss by leakage. Since the actual potassium content of the erythrocytes did not appear to have dropped and since there was no hemolysis, it was inferred that the potassium in the plasma had been increased by diffusion from extravascular sources.

The need for taking blood promptly after death, if reliance is to be placed on analytical results, is illustrated by the rise in plasma potassium that occurred during the first hour post mortem. At death, the plasma concentration of potassium was 9.3 milliequiv/l, whereas 1 hour later it was 16.8. Although there is no evidence in the data presented in Table 19 as to the source of this increment, other observations indicated that both leakage from red blood cells and diffusion from extravascular tissues may cause a post-mortem rise in plasma potassium. So far as the significance of this experiment in providing control data is concerned, it is apparent that a twofold rise in plasma potassium may occur as a result of severe systemic anoxia.

In order to correlate chemical data with known degrees of cutaneous hyperthermia, it was decided to submerge animals in hot water rather than expose them to hot air. By the former method, the temperature of the surface of the skin could be controlled with greater precision than was possible by the latter.

The experimental procedure that was followed in submerging animals in hot water is described in detail in Section 17.11. The animals were anesthetized with pentobarbital sodium and between 60 and 75 per cent of the total body surface was raised to the desired level. The effects on the blood of exposing four pigs to water at 47 C and eight pigs to water at 75 C are shown in Table 20.

Exposure at 47 C: Although all these animals developed an acute and rapidly fatal systemic hyperthermia, none showed a rise in plasma potassium significantly greater than that which may result from anoxia independently of hyperthermia. In none of these was the magnitude of the increase comparable to that which was observed in some of the severely burned animals reported in Section 17.9.

In the first two animals, it appeared that the potassium increase in the plasma was derived from extravascular sources. In the third animal the increase was due to leakage in only one sample. In the fourth animal it may have been due in part to leakage from

intact erythrocytes, and in part to diffusion from extravascular tissue. Cutaneous hyperthermia of the kind produced in these animals did not result in a significant amount of intravascular hemolysis.

TABLE 20. Effects on the blood of exposing pigs to hot water.

Pig No.	Time min	Thermal exposure C	Body temp C	Time of death	Blood samples time taken	Volume packed cells	Hemoglobin		Potassium in red cells milliequiv/l	Potassium in plasma — milliequiv/l		Potential increment from hemolysis	Increment from other sources than hemolysis
							Hemo-globin in cells g/100 ml	% hemol-ysis		Total	Change		
877	Control		34.3		Control	32	29	0	145	3.8		...	...
	0	Started } 47											
	10		...	..	10 min	33	30	0.	158	6.2	+2.4	0	2.4
	14		...	..	14 min	33	30	0.	154	6.9	+3.1	0	3.1
	24		44.3	..	24 min	31	32	0.	158	8.2	+4.4	0	4.4
	26	Stopped	...	+	...	..	..	...	...	...		...	...
1057	Control		37.0	..	Control	35	35	0.1	115	4.4		...	...
	0	Started } 47											
	20		...	..	20 min	36	35	0.0	125	7.0	+2.6	0	2.6
	36	Stopped	45.5	+	36 min	36	35	0.2	120	10.2	+5.8	0.2	5.6
1056	Control		37.8	..	Control	33	37	0.1	118	4.7		...	...
	0	Started } 47											
	10		...	..	10 min	33	36	0.1	114	5.9	+1.2	0	1.2
	15		...	..	15 min	35	35	0.1	113	7.2	+2.5	0	2.5
	34		...	..	34 min	36	35	0.2	118	7.1	+2.4	0.1	2.3
	45	Stopped	45.5	+	...	..	..	...	...	...		...	...
923	Control		?	..	Control	48	33	0.0	?	3.8		...	...
	0	Started } 47											
	13		...	..	13 min	47	44	0.1	124	5.5	+1.7	0.1	1.6
	23		...	..	23 min	46	46	0.2	120	5.5	+1.7	0.2	1.5
	34		...	..	34 min	55	32	0.1	113	6.2	+2.4	0.2	2.2
	42		...	..	42 min	55	32	0.1	112	6.5	+2.7	0.2	2.5
	47		...	..	47 min	56	33	0.1	?	7.5	+3.7	0.2	3.5
	50	Stopped	?	+	...	..	..	...	...	...		...	...
899	Control		37.4	..	Control	38	34	0.4	139	3.6		...	...
	0	Started } 75											
	1		...	..									
	5		...	..	5 min	48	31	3.6	109	10.2	+6.6	3.7	2.9
	16		...	..	16 min	37	33	8.6	117	6.9	+3.3	6.5	...
	46		...	..	46 min	39	32	7.5	118	4.2	+0.6	6.2	...
	76	Stopped	39.2	..	76 min	37	35	6.7	122	7.4	+3.8	5.2	...
918	Control		36.6	..	Control	34	44	0.0	131	3.7		...	...
	0	Started } 75											
	3		...	..									
	4		...	..	4 min	51	30	2.5	98	11.0	+7.3	2.6	4.7
	11		...	..	11 min	45	42	4.4	110	9.5	+5.8	4.2	1.6
	17		...	..	17 min	44	35	5.9	102	9.5	+5.8	5.1	0.7
	37		40.6	..	37 min	40	48	5.6	103	9.4	+5.7	4.0	1.7
	55	Stopped	...	+	...	..	..	...	...	...		...	...
919	Control		...	..	Control	45	33	0.8	118	4.2		...	...
	0	Started } 75	37.1	..	...	..	..	...	...	...		...	...
	4		...	..	4 min	56	29	7.8	81	25.5	+21.3	8.6	12.7
	5		...	..	...	..	..	...	...	...		...	...
	8		...	..	8 min	47	26	25.5	67	21.4	+17.2	20.2	...
	10		...	..	10 min	40	32	22.2	?	18.3	+14.1	?	...
	14		...	..	14 min	35	37	23.1	77	17.0	+12.8	12.6	...
	17		44.3	..	17 min	33	31	30.1	72	17.5	+13.3	15.2	...
	18		...	+	...	..	..	...	...	...		...	...
913	Control		38.6	..	Control	26	37	0.0	116	3.5		...	...
	0	Started } 75											
	2		...	..	2 min	35	32	12.3	103	14.2	+10.7	7.7	3.0
	6		...	..	6 min	32	33	24.5	96	17.7	+14.2	14.7	...
	7	Stopped	...	..	...	..	..	...	...	...		...	...
	8		40.8	+	8 min	30	32	25.3	111	17.4	+13.9	16.0	...
907	Control		37.3*	..	Control	42	34	0.6	125	3.5		...	...
	0	Started } 75											
	8		...	..	8 min	53	31	2.7	100	17.4	+13.9	3.1	10.8
	10	Stopped	42.5*	+	...	..	..	...	...	...		...	...

* Right heart temperature.

SECRET

TABLE 20 (Continued).

Pig No.	Time min	Thermal exposure C	Body temp C	Time of death	Blood samples time taken	Volume packed cells	Hemoglobin		Potassium in red cells milliequiv/1	Potassium in plasma — milliequiv/1		Potential increment from hemolysis	Increment from other than hemolysis
							Hemo-globin in cells g/100 ml	% hemol-ysis		Total	Change		
910	Control	75	36.8	..	Control	..	?	?	...	3.0		...	...
	0												
	2		...	..	2 min	..	?	?	...	19.1	+16.1	...	...
	5		...	..	5 min	..	?	?	...	18.1	+15.1	...	...
	7		...	..	7 min	..	?	?	...	24.0	+21.0	...	...
	13		...	+	...	..	..	...	...	...		...	...
	14	Stopped	43.7	..	14 min	..	..	...	...	17.3	+14.3	...	...
908	Control	75	?	..	Control	32	?	?	106	3.8		...	...
	0												
	4		...	..	4 min	?	?	?	?	16.7	+12.9	...	...
	9		...	..	9 min	33	?	?	98	18.5	+14.7	...	...
	11		...	..	11 min	32	?	?	90	17.1	+13.3	...	...
	14	Stopped	?	+	...	..	..	...	...	...		...	...
912	Control	75	36.0	..	Control	33	37	0.0	125	4.1		...	...
	0												
	1		...	..	1 min	45	31	1.9	102	16.7	+12.6	1.6	11.0
	4		...	..	4 min	33	37	19.2	?	?	?	?	...
	5		...	..	5 min	34	34	24.2	100	16.4	+12.3	16.5	...
	10		...	..	10 min	40	31	19.9	85	16.4	+12.3	14.2	...
	14	Stopped	43.1	+	...	..	..	...	...	...		...	...

Exposure at 75 C: The chemical changes in this group were of a different order of magnitude from those observed in animals exposed at 47 C. All animals exposed for 5 minutes, or longer, at 75 C developed plasma potassium levels in excess of 16 milliequiv/l. In most instances, such levels were reached during the first few minutes of exposure and were either maintained or increased as the period of exposure was prolonged. If the pig survived for more than a few minutes after the termination of the exposure, there was a slow decline in plasma potassium concentration. Thus, in animal 919 the plasma potassium rose from 4.2 to 25.5 milliequiv during the first 4 minutes of exposure, and during the next 4 minutes declined to 17.4.

The rapidity with which an excessively high plasma potassium level may be lowered by extravascular diffusion is indicated by the discrepancies that were observed between estimated increments by hemolysis and total amounts present. Thus, it may be seen in the case of pig 913 that with an increment by hemolysis of 7 milliequiv/l between the 2- and 6-minute samples, the actual plasma level rose by only 3.5 milliequiv. Similarly, in pig 912 the increment by hemolysis between the 1- and 5-minute samples was 14.9 milliequiv/l, whereas the total plasma potassium actually changed from 16.7 to 16.4 during this period.

In most of the animals exposed at 75 C, there was some increase in the volume of packed cells. The

comparison of cell volume and hemoglobin content indicated that most, if not all, of the early increase in cell volume was due to swelling of erythrocytes rather than to loss of plasma or mobilization of cells from storage depots.

It is of interest to note that plasma hemoglobin values as high as 24 per cent hemolysis were observed as early as 5 minutes after the onset of cutaneous hyperthermia. It was estimated that during this period the temperature in the vicinity of the most superficial blood vessels probably rose to approximately 70 C.

Chemical changes in the blood of dogs caused by cutaneous hyperthermia: It was inferred from the foregoing experiments on pigs that most of the potassium responsible for these potentially fatal plasma levels either leaked out of intact red blood cells or escaped from hemolyzed cells. If this inference is correct, fatal hyperpotassemia due to cutaneous hyperthermia would occur only in animals having a high concentration of potassium in the erythrocytes, such as man or pig. Its occurrence could not be expected in an animal having a low cellular concentration of potassium, as is the case in dog's blood.

To test this assumption, samples of blood were taken from each of five dogs before and during immersion in hot water. The results of these experiments are shown in Table 21.

The animals were exposed at temperatures ranging between 55 and 75 C until death occurred. The high-

TABLE 21. Changes in blood of dogs caused by immersion in hot water.

Dog No.	Time in min	Thermal exposure C	Body temp C	Time of death	Blood samples time taken	Volume packed cells	Hemoglobin in cells g/100 ml	Hemoglobin in plasma % hemolysis	Potassium in red cells milliequiv/l	Potassium in plasma milliequiv/l
931	Control	Started } 55 Stopped }	35.4	..	Control	35	37	0	9.4	2.8
	0		...	..	5 min	41	36	0	8.1	5.2
	5		...	..	13 min	57	32	0	10.7	4.7
	13		41.4	..	21 min	57	33	0	11.2	6.9
	21		...	+		..	..	...	...	..
930	Control	Started } 60 Stopped }	36.9	..	Control	49	34	0.1	4.3	4.0
	0		...	..	5 min	66	27	17.9	6.4	3.3
	5		...	..	8 min	65	28	20.2	5.5	4.7
	8		39.1	..	11 min	62	28	23.8	6.1	5.3
	11		...	+		..	..	...	...	..
929	Control	Started } 75 Stopped }	37.2	..	Control	49	34	0.3	6.3	3.9
	0		...	..	3 min	57	29	26.1	7.0	4.8
	3		...	..	9 min	42	37	31.8	5.7	6.1
	9		44.1	..	13 min	39	34	35.8	7.9	8.2
	13		...	+		..	..	...	...	..
922	Control	Started } 75 Stopped }	37.9	..	Control	42	35	0.2	8.8	3.1
	0		...	..	3 min	47	30	22.9	8.9	5.8
	3		...	..	7 min	47	30	29.5	12.6	6.4
	7		...	..	10 min	43	29	33.5	7.9	5.8
	10		39.3	+	15 min	45	30	31.4	8.0	6.8
934	Control	Started } 75 Stopped }	34.6*	..	Control	41	35	0.1	5.6	3.1
	0		...	..	25 min	40	34	31.9	6.5	6.9
	25		43.5*	+						

* Right heart temperature.

est potassium concentration observed in the erythrocytes in control samples of blood from these animals was 9.4 milliequiv/l, in contrast to the pig, whose erythrocyte concentrations ranged between 106 and 145 milliequiv/l. The greatest potassium increase that occurred in the plasma of the dogs that died as a result of cutaneous exposure to heat was from 3.9 to 8.2 milliequiv/l.

The increments to the plasma potassium that were observed in these animals could not be accounted for by loss of potassium from the erythrocytes. The potassium content of the red blood cells of the dogs characteristically rose during exposure in contrast to the loss of potassium that occurred from the erythrocytes of the pig. As in the case of the pig, there was severe intravascular hemolysis in animals exposed at 75 C until death occurred.

It can be inferred, therefore, that the development of a potentially fatal level of hyperpotassemia following cutaneous exposure to heat results from the rapid release of potassium from thermally injured red blood cells and that a high erythrocyte content of potassium is essential to its occurrence.

17.10.4 *In Vitro* Effects of Heat on Pig's Blood

It was thought that more precise information regarding the reciprocal relationships of temperature, time, and the release of potassium from erythrocytes could be obtained by heating samples of pig's blood *in vitro*.

Heart's blood was collected from normal pigs by cardiac puncture in a heparinized syringe, where it was mixed and then discharged into heparinized glass-stoppered vials. One vial was kept at room temperature as a control; the others were strapped to a mechanical mixer and immersed in a constant temperature bath. Exposure temperatures ranged between 44 and 63 C; during exposure the blood was mechanically decanted from one end of the vial to the other at a rate of six times per minute. It required approximately 2 minutes for the temperature of the blood to reach that of the water bath. As soon as a sample was removed from the water bath, it was immediately cooled in ice water and analyzed.

It is apparent that there was a progressive increase in the rate at which potassium passed out of the

TABLE 22. *In vitro* effects of heat on pig's blood.

Specimen	Temp C	Time in minutes	Volume packed cells	Hemoglobin in cells g/100 ml	Hemoglobin in plasma % hemolysis	Potassium in red cells milliequiv/l	Potassium in plasma — milliequiv/l			
							Total	Change	Increment from hemolysis	Increment from leakage
1-947	Control	Control	30	34	0.1	105	3.2	...	..	...
	40	15	30	34	0.1	113*	3.5	+0.3	..	0.3
	..	30	30	32	0.3	114*	3.5	+0.3	0.1	0.2
	..	60	30	34	0.1	102	3.8	+0.6	..	0.6
2-949	Control	Control	32	33	0	99	3.2	...	..	...
	44	15	31	33	0.1	107*	3.9	+0.7	..	0.7
	..	30	31	34	0.1	102*	4.0	+0.8	..	0.8
	..	60	31	33	0.3	97	4.8	+1.6	0.1	1.5
3-949	Control	Control	31	34	0	104	4.6	...	..	...
	48	15	32	31	0.1	101	7.5	+2.9	..	2.9
	..	30	32	32	0.1	90	9.2	+4.6	..	4.6
	..	60	32	29	0.4	90	11.0	+6.4	0.1	6.3
4-950	Control	Control	33	32	0.0	109	4.3	...	..	...
	51	15	35	31	0.8	96	10.2	+5.9	0.4	5.5
	..	30	34	34	0.5	98	11.8	+7.5	0.2	7.3
	..	60	36	31	0.7	92	10.7	+6.4	0.4	6.0
5-950	Control	Control	34	35	0.1	120	4.2	...	..	...
	52	15	35	34	0.8	103	10.0	+5.8	0.5	5.3
	..	30	35	32	2.7	101	10.4	+6.2	1.5	4.7
	..	60	36	32	2.7	100	10.9	+6.7	1.6	5.1
6-947	Control	Control	31	33	0.1	109	4.2	...	..	...
	55	15	40	28	1.3	85	7.5	+3.3	0.7	2.6
	..	30	37	30	5.7	87	12.1	+7.9	3.0	4.9
	..	60	37	30	9.6	71	18.5	+14.3	4.4	9.9
7-1052	Control	Control	38	33	0.0	119	3.6	...	..	...
	60	5	48	28	1.4	83	12.6	+9.0	1.2	7.8
8-1052	Control	Control	36	35	0.1	121	4.1	...	..	...
	61	5	45	28	3.5	76	20.8	+16.7	2.4	14.3
9-1052	Control	Control	34	36	0.0	122	3.9	...	..	...
	62	5	33	34	11.7	69	30.8	+26.9	4.5	22.4
10-1052	Control	Control	36	36	0.1	121	4.1	...	..	...
	63	5	26	34	31.6	58	40.2	+36.1	9.6	26.5

* These values must be due to analytical errors.

erythrocytes and into the plasma of the blood as its temperature was raised (Table 22). The amounts of the plasma increment at the end of 1 hour's exposure at 40, 44, 48, 51, 52, and 55 C were respectively 0.6, 1.6, 6.4, 6.4, 6.7, and 14.3 milliequiv/l. At the lower temperatures (51 C and under), the increments were due almost entirely to leakage from intact cells. At the end of 30 minutes of exposure at 52 and 55 C, the proportion of the plasma increment contributed by hemolysis was 24 and 38 per cent, respectively.

Unequivocal evidence of swelling of erythrocytes was first observed at 55 C, although there may have been some swelling in all specimens exposed for more than 30 minutes at 48 C and higher.

The rate of change in the blood was much more rapid during exposures at 60 C and higher. In these experiments the blood remained in the bath for only 5 minutes and the actual time during which it was at the temperature of the water was approximately 3 minutes. The rise in plasma potassium after such

brief periods at 60, 61, 62, and 63 C was, respectively, 9.0, 16.7, 26.9, and 36.1 milliequiv/l. The blood was totally hemolyzed at 65 C.

Not until blood was heated at 60 or higher in a test tube were the observed increases in plasma potassium comparable with those that occurred in living pigs after cutaneous exposures at 75 C. This is not to imply that the effects of hyperthermia on blood in a test tube are necessarily similar to those effects in a living animal. Attention has already been called to the fact that asphyxia without rise in temperature may cause hyperpotassemia in a living animal. Although the mean temperature of the blood of a living pig is never raised to 60 C, most or all of its blood may in the course of its circulation through the overheated dermis be brought to a much higher temperature than would be recorded by a rectal thermometer or intracardiac thermocouple. It will be recalled from the calculations made in Section 17.3 that the superficial portion of the dermis of a living pig

reaches a temperature of 60 C within a second after the surface of the skin has been brought to 75 C. It would appear quite possible then that the temperature of most or all of the blood of an animal that had received an extensive cutaneous exposure to water at 75 C for as long as 5 minutes would be raised briefly during its passage through the subcutaneous tissue to the neighborhood of 60 C.

Not until the temperature of the bath was raised to 62 C did a 5-minute exposure of blood in a test tube result in hemolysis comparable with that observed in living pigs exposed at 75 C.

Attention has already been directed to the fact that unequivocal swelling of erythrocytes was first observed in a test tube after a 15-minute exposure at 55 C. So far as could be judged by the hemoglobin-hematocrit ratios, swelling of erythrocytes continued through 61 C, beyond which it was not observed.

17.10.5 Summary

These experiments have established that severe and extensive cutaneous burning may result in a rapid rise in plasma potassium to levels ordinarily considered incompatible with life. Such levels are obtained when a large proportion of body surface is maintained at 75 C for more than a few minutes. That lower surface temperatures may also be responsible for fatal hyperthermia is suggested by the fact that potassium is released rapidly from blood cells *in vitro* at temperatures of 60 C or over. In part because of the slowness with which potassium is released at lower temperatures and in part because of the rapidity with which excess potassium leaves the blood stream, it is not likely that thermal exposures of insufficient intensity to cause severe cutaneous burning could cause sufficient damage to the erythrocytes to produce dangerously high plasma levels.

In vitro experiments on pig's blood indicate that rapid leakage of potassium from erythrocytes occurred when its temperature was raised over 60 C and that rapid hemolysis occurred when its temperature was raised above 62 C. Leakage was accompanied by swelling at temperatures ranging between 55 and 61 C. Above that temperature, so far as could be judged by the hemoglobin content of cells, rapid release of potassium occurs without cell swelling.

It was demonstrated that leakage from and lysis of red blood cells were the principal sources of the potassium increments of plasma. At the lower temperatures (47 C *in vivo* and 48 C *in vitro*) hemolysis was negligible. The increase in plasma potassium

in vivo at these temperatures was due either to diffusion from extravascular sources or to leakage from erythrocytes. It was obvious in the lower-temperature *in vitro* experiments that leakage from erythrocytes was the only source of the plasma increment. Although leakage alone could be sufficient to account for potentially fatal plasma levels (in excess of 16 milliequiv/l), no such increases were observed without accompanying hemolysis. When blood was heated *in vitro* leakage contributed more than hemolysis to the attainment of such levels. In thermal exposures *in vivo* of sufficient duration and intensity to produce comparable levels, hemolysis was the more important factor.

17.11 PHYSIOLOGICAL DISTURBANCES FROM EXCESSIVE HEAT^j

17.11.1 Introduction

In Section 17.9 of this chapter, attention was called to the fact that acute hyperthermic circulatory failure in some animals was accompanied by, and undoubtedly contributed to by, large increases in the potassium concentration of the plasma. An investigation of the circumstances in, and the sources from which, thermally induced rises in plasma potassium occur has been described in Section 17.10.

Although it appeared that central circulatory failure caused by hyperpotassemia was one of the mechanisms responsible for death incident to cutaneous exposure to heat, it was apparent that this was not the sole cause of death during hyperthermia. The following investigations² were undertaken for the purpose of determining the precise nature of the various kinds of circulatory disturbances which may result from cutaneous exposure to excessive heat.

The acute physiological disturbances caused by systemic hyperthermia have attracted the attention of a number of investigators. Heymans²⁵ injected methylene blue into dogs anesthetized with chloralose. This produced a gradually mounting rectal temperature which reached the lethal level of 43.7 to 44.8 C in 1 to 1½ hours. The heart rate rose gradually from 90–120 to 300–330 per minute. At first the respirations were deep and rapid (less than 200 per minute); after the temperature had risen to 41.5–43.5 C they became very shallow and even more rapid (over 300 per minute). Systolic pressure rose and diastolic pressure fell. Respiration almost always failed first, and artificial respiration enabled the

^j By Albert Roos.

heart to continue for a longer time. Reflexes persisted up to the time of respiratory standstill. Uyeno⁴⁵ produced hyperthermia in cats, anesthetized with urethane, by exposing them to water of 41–42 C or to a high environmental air temperature. During the 30 minutes of exposure the rectal temperature rose from 35 to 39 C. There was little increase in heart rate, but a pronounced rise in minute-volume output. Shortly after exposure the respiratory rate increased to an average of 200 per minute. This breathing was very shallow (tidal air 2–3 cc per minute) and sometimes resulted in a 29 per cent drop in arterial oxygen saturation. Cheer¹³ placed dogs anesthetized with morphine and barbitol in a cabinet heated by electric light bulbs. In 2 to 3 hours a lethal (rectal?) temperature of 43–45 C was reached. The heart rate increased progressively until a temperature of 42–44 C was reached, when the heart slowed rather suddenly. Before this stage electrocardiographic abnormalities were limited to slight abbreviation of the *PR* interval, slight changes in the *QRS* complex, and inversion of the *T* wave. The terminal bradycardia was due to the development either of nodal rhythm or of various other types of ventricular rhythm. Systolic and diastolic pressures remained fairly constant up to 41 C, then both dropped, the former more than the latter. The respiratory rate also increased. Respiratory standstill usually occurred before cardiac arrest, vagotomy delaying respiratory failure. A progressive decrease of the blood carbon dioxide was found associated with slight alkalosis and rise of oxygen content, which were ascribed to the increased pulmonary ventilation. From the same laboratory, Wiggers and Orias⁴⁷ reported observations on the effects of short radio waves on dogs. The cardiac acceleration, increase in rate and depth of the respiration, and primary failure of the respiration were identical with the findings of Cheer.¹³ However, instead of a decrease in blood pressure, a rise of systolic and diastolic pressure was observed which progressed until death.

Clinical observations on the effect of hyperthermia were made by Ferris *et al.*¹⁵ Patients with heat stroke whose rectal temperatures varied from 39.9 to 44.0 C exhibited a hot dry skin, a normal or elevated systolic pressure, which dropped to low levels only in the terminal stage, and venous pressures of from 2 to 12 cm of saline. Their respiratory rate was 28 to 50 per minute. Of 29 patients (all comatose) whose temperatures exceeded 41.5 C, 17 died; all others recovered.

Attempts to analyze the disturbances observed in the intact organism by elevating the temperature in one organ have been made since 1872. Fick¹⁶ heated the blood as it passed through the carotid arteries of the dog and noticed marked hyperpnea without change in heart rate or blood pressure. Cyon¹⁴ isolated the circulation of a dog's head. Perfusion of the head with heated blood produced bradycardia and a drop in blood pressure. Kahn²⁸ warmed the carotid arteries of unanesthetized dogs without producing a rise in rectal temperature. He observed the development of tachycardia and a moderate rise in blood pressure. Moorhouse³⁶ heated the carotids and simultaneously cooled the jugular veins in dogs. This resulted in tachycardia, rarely preceded by bradycardia, ascribed respectively to increased sympathetic and vagal activity. Coincidentally, tachypnea and peripheral vasodilatation were observed. Heymans and Ladon²⁶ severed all connections except the vagal nerves between head and trunk of dogs anesthetized with chloralose. Artificial respiration was applied and the circulation in the head maintained by connecting it to a donor dog. The sublingual temperature of the preparation rose to 45 C in 1½ hours. There was no change in the heart rate which had risen to 160 after severance of the cervical cord. The head exhibited a progressive and pronounced increase in respiratory rate which persisted until a sublingual temperature of 45 C was reached, when the rate rapidly decreased and the reflexes of the head, which had been active up to that time, disappeared.

The effect of hyperthermia on the heart was investigated by Knowlton and Starling,³¹ using the innervated heart-lung preparation perfused with heated blood. From 26 C to approximately 45 C the heart rate was a linear function of the blood temperature, the rate at 45 C being 180 per minute. Above this temperature marked slowing occurred and the heart soon stopped. Arrhythmias occurred above 40 C.

To summarize these data, it can be said that in the dog the highest rectal temperature compatible with life lies between 43 and 45 C, when this temperature is reached in 1 to 3 hours. Respiratory failure often seems to precede circulatory failure. Tachypnea, tachycardia, and peripheral vasodilatation seem to be, in part at least, of cerebral origin.

The physiological changes of rapidly developing hyperthermia leading to death within half an hour have not been heretofore studied. As high environ-

TABLE 23. Rectal temperature, arterial pressure, and electrocardiogram of 12 pigs immersed in hot water.

A — Normal sinus rhythm (normal rate, tachycardia, or bradycardia).

Normal duration of *QRS* complex.A' — First or second degree A-V block. Normal duration of *QRS* complex.A'' — Complete A-V block. Normal duration of *QRS* complex.

B — Slight

BB — Moderate

BBB — Pronounced

} Widening of *QRS* complex without *P* wave.

BBB — Can often be interpreted as ventricular fibrillation.

Time		Rectal	Arterial	ECG	Time		Rectal	Arterial	ECG			
min	sec	temp C	pressure (mm Hg)		min	sec	temp C	pressure (mm Hg)				
Pig 876 (7.7 kg) 48 C. Died after 26.5 min.					Pig 897 (16.4 kg) 47 C. Curare. Died after 56 min.							
		Control	34.3	118	A			Control	37.9	146	...	
16	..		44.0	66	A	24	..		43.5	146	A	
24	30		45.2	42	A	47	..		44.0	90	A	
26	30		45.7	76	BB	55	..		...	36	A'	
Pig 875 (6.4 kg) 48-50 C. Died after 35 min.					Pig 946 (9.5 kg) 47 C for 23 min. Curare. Died after 42 min.							
		Control	35.0	130	A			Control	40.1	82	A	
27	30		42.2	64	A	17	..		43.0	120	A	
29	..		42.8	64	A	26	..		44.4	40	A	
34	15		43.7	26	A	34	30		44.6	26	A	
Pig 878 (12.0 kg) 47 C. Died after 50 min.					Pig 944 (10.4 kg) 47 C for 25 min. Died after 99 min.							
		Control	...	110	A			Control	38.1	108	A	
29	..		...	70	A	14	..		43.5	120	A	
37	20		...	50	A	26	..		45.4	100	A	
49	30		44.9	30	A	37	..		44.1	90	A	
Pig 879 (11.8 kg) 44-47 C. Died after 106.5 min.					Pig 867 (7.3 kg) 64-65 C. Died after 15 min.							
		Control	36.8	106	A			Control	...	146	A	
33	..		43.1	54	A	5	30		...	72	A	
Out of hot bath* from 33.5 to 48.5 min.							10	30		...	72	A
49	53		42.0	116	...	15	..		46.0	12	BB	
79	30		44.1	86	A	Pig 872 (7.3 kg) 64-65 C. Died after 11 min.						
105	..		44.5	14	A			Control	...	150	A	
Pig 895 (18.0 kg) 49 C. Curare. Died after 32 min.							7	..		...	50	A
		Control	37.8	148	A	10	30		...	50	BB	
15	..		41.9	172	A	10	45		...	40	BBB	
25	30		43.7	76	A	Pig 871 (9.1 kg) 70-73 C. Died after 12 min.						
31	30		44.0	10	A'†			Control	...	100	...	
Pig 943 (8.3 kg) 47 C. Curare. Died after 36 min.							5	30		...	74	A
		Control	37.7	126	A	6	10		...	74	BB	
17	..		42.6	126	A	9	30		...	54	BBB	
29	..		44.5	136	A	12	..		44.5	24	BBB	

* Skin temperature lowered by exposure to cool water between two episodes of cutaneous hyperthermia.

† Occasional ventricular extra-systole.

mental temperatures are needed for such experiments, the results are necessarily complicated by the damaging effect of heat on the skin directly. Moreover, these high temperatures will produce damage to the red blood cells that are circulating in the small vessels of skin and underlying tissues.⁴²

17.11.2 Experimental Procedure

Young pigs weighing from 6.4 to 18 kg and adult dogs weighing from 7.4 to 8.5 kg were used as experimental animals. They were anesthetized with pentobarbital sodium (32 mg/kg intraperitoneally), shaved, and tied to a wooden animal board. This

was lowered into a galvanized iron tank (92x46x41 cm). The head of the board rested on a metal bar in the tank, so that it was slightly higher than the foot. A similar tank, placed on a high table, partly projected over the former. This tank was filled with water steam-heated to the desired temperature. In the bottom of the projecting part was a circular opening 13 cm in diameter that could be closed with a heavy rubber and metal stopper, resulting in full immersion in 8 to 10 seconds. During immersion, the temperature of the water, which was continuously stirred, was kept within narrow limits by intermittent introduction of steam. Drainage of

TABLE 24. Rectal temperature, arterial pressure, electrocardiogram, hematocrit, and hemoglobin and potassium content of plasma and of red blood cells of 15 pigs immersed in hot water.

A — Normal sinus rhythm (normal rate, tachycardia or bradycardia).

Normal duration of *QRS* complex.A' — First or second degree A-V block. Normal duration of *QRS* complex.A'' — Complete A-V block. Normal duration of *QRS* complex.

B — Slight

BB — Moderate

BBB — Pronounced

BBB — Can often be interpreted as ventricular fibrillation.

} Widening of *QRS* complex without *P* wave.

Time		Rectal	Arterial	ECG	K plasma milliequiv/l	Time		Rectal	Arterial	ECG	K plasma milliequiv/l
min	sec	temp C	pressure			min	sec	temp C	pressure		
Pig 877 (7.0 kg) 47 C. Died after 26 min.						Pig 905 (12.7 kg) 75 C. Curare. Died after 23 min.					
Control		34.3	96	A	3.8	Control		...	94	A	4.8
10	20	41.6	136	A	6.2	16	30	41.6	78	BB	...
14	5	42.5	112	A''	6.9	22	40	42.1	32	BBB	17.3
24	10	44.3	56	A''	8.2	Pig 921 (16.8 kg) 75 C. Curare. Died after 27 min.					
Pig 923 (13.6 kg) 47 C. Died after 50 min.						Control		...	122	A	3.2
Control		...	116	A	3.8	3	30	...	66	A	5.1
13	15	...	146	A	5.5	8	..	...	58	BB	11.6
22	30	...	146	A	5.5	18	..	...	36	B	11.9
34	15	...	102	A	6.2	26	45	...	28	B	10.2
42	..	...	56	A	6.5	Pig 906 (13.0 kg) 70-75 C. Curare. Died after 70 min.					
46	33	...	66	A	7.5	Control		38.6	102	A	4.0
Pig 1057 (8.0 kg) 47 C. Died after 36.5 min.						10	50	41.4	112	BBB	...
Control		37.0	...	A	4.4	16	35	42.3	62	BB	17.4
19	50	...	...	A	7.0	25	20	43.0	92	BBB	15.2
36	15	...	...	A	10.2	44	35	44.6	72	BB	13.3
36	30	45.5	...	O	...	46	40	44.8	46	A	...
Pig 1056 (7.0 kg) 47 C. Died after 44.5 min.						48	29	45.0	46	BB	...
Control		37.8	...	A	4.7	65	..	46.8	46	BBB	...
9	30	...	...	A	5.9	Pig 913 (8.2 kg) 75 C for 6.5 min. Died after 7.5 min.					
15	7	...	...	A	7.2	Control		38.6	100	A	3.5
34	..	...	...	A	7.1	2	25	37.9	100	B	14.2
44	30	45.5	...	O	...	6	15	40.5	50	BBB	17.7
Pig 910 (9.5 kg) 72-75 C. Died after 12.5 min.						7	45	40.8	15	O	17.4
Control		36.8	148	A	3.0	Pig 919 (9.1 kg) 75 C for 5 min. Died after 18 min.					
2	15	40.7	100	A	19.1	Control		37.1	138	A	4.2
4	40	40.7	86	BB	18.1	4	15	41.1	78	BB	25.5
7	20	41.5	74	BBB	24.0	7	45	42.3	28	A	21.4
13	52	43.7	10	O	17.3	10	10	43.2	26	A	18.3
Pig 912 (10.0 kg) 72-75 C. Died after 14 min.						14	..	44.2	30	B	17.0
Control		36.0	88	A	4.1	16	45	44.3	14	B	17.5
1	20	35.4	154	A	16.7	Pig 918 (8.7 kg) 75 C for 3 min. Died after 55 min.					
3	35	37.0	98	BB	...	Control		36.6	70	A	3.7
5	7	37.1	74	BB	16.4	4	25	38.7	56	A	11.0
9	45	40.8	74	BBB	16.4	11	..	39.7	62	A	9.5
13	40	43.1	30	BBB	...	17	5	40.3	70	A	9.5
Pig 908 (9.1 kg) 75 C. Died after 13.5 min.						37	..	40.6	70	A	9.4
Control		...	96	A	3.8	Pig 899 (13.6 kg) 75 C for 1 min. Sacrificed after 77 min.					
3	40	...	96	BB	16.7	Control		37.4	142	A	3.6
8	55	...	60	BBB	18.5	5	15	40.5	30	A	10.2
11	10	...	52	BBB	17.1	16	5	40.5	76	A	6.9
Pig 907 (10.4 kg) 75 C. Died after 10 min.						45	45	40.3	76	A	4.2
Control		37.1	116	A	3.5	76	..	39.2	76	A	7.4
..	..	37.3*	...	...	...						
6	..	39.0	48	BBB	...						
..	..	42.7*	...	...	...						
7	30	39.2	32	BBB	17.4						
..	..	42.5*	...	...	...						

* Right heart temperature.

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the water and termination of exposure could also be accomplished in 8 to 10 seconds. Temperatures ranging from 44 to 75 C were used.

Previous to exposure, all animals were heparinized (3 mg/kg intravenously). Because of spasmodic closure of the glottis on immersion, a tracheal cannula was inserted. The carotid pressure was recorded with a mercury manometer. The right auricular pressure was measured by means of a rubber catheter introduced into the superior vena cava or right auricle by way of the external jugular vein and connected with a water manometer. The level of the right auricle as determined by opening the chest at the end of the experiment was taken as the point of reference. In pigs the hydrostatic pressure did not influence the auricular pressure. In dogs immersion resulted in a considerable rise in recorded auricular pressure, so that only changes occurring during exposure could be compared. Pneumograms were obtained by means of a copper cannula thrust between the ribs into the pleural space and connected by means of a rubber tube to a writing tambour. In other experiments, a tracheal cannula provided with a sealed-in side tube connected to the tambour was used. Electrocardiograms were taken with an amplifier type of electrocardiograph. It was only possible to take the first standard lead, as the hind legs of the animal were under water. In some experiments, curarized animals were used and artificial respiration was applied throughout the exposure. Intocostin (Squibb) 1 mg/kg diluted with saline was slowly injected intravenously. The side reactions were limited to a short (20 to 30 seconds) period of mild excitation. The drug had no effect on the arterial pressure. A second smaller dose usually had to be given 20 to 40 minutes later. A Palmer respiration pump for small animals, which allows the air to escape spontaneously on expiration, was used. When venous pressures were recorded the animals were immersed in such a manner that most of the anterior thorax remained above the water level. This was sufficient to abolish artifacts produced by the increased resistance to the inflow of air. Temperatures were recorded with a thermocouple introduced to a depth of 7 to 9 cm into the rectum, which had been cleaned by repeated enemas. The anus was closed around the couple. In three experiments, heart temperatures were also recorded by means of a thermocouple introduced through the external jugular vein into the right auricle. In some experiments only initial and final rectal and final heart temperatures were meas-

ured with a sensitive thermometer. In a considerable number of animals blood was withdrawn from the jugular vein both before and during exposure for the determination of the hematocrit and of hemoglobin and potassium content of red cells and plasma (Section 17.10). In most instances, immersion was continued until death. In some experiments exposure was temporarily interrupted, and, in a few cases, immersion was terminated at a time when the animal was still living.

In addition to these observations, three pigs were infused with an isotonic (1.12 per cent) solution of KCl. Frequent electrocardiograms (lead I or II) were taken. In one of these pigs, the arterial and right auricular pressure and respirations were also recorded. The latter animal received the solution in the subclavian vein, the other two in the jugular vein. Blood samples for the determination of potassium were taken from the carotid artery.

17.11.3 Results of Experiments

In Table 23 are shown the results of 12 experiments in which pigs were exposed for varying periods of time at temperatures ranging between 44 and 73 C. Changes in rectal temperature, arterial pressure, and electrocardiogram are indicated.

In Table 24 are shown the results of 15 experiments in which pigs were exposed at temperatures ranging between 47 and 75 C. The changes that occurred in the potassium concentration of the plasma are indicated in relation to changes in rectal temperature, arterial pressure, and electrocardiogram.

In Table 25 are shown the results of 5 experiments in which dogs were exposed for varying periods of time at temperatures ranging between 55 and 75 C. The changes that occurred in the potassium concentration of the plasma are indicated in relation to changes in rectal temperature, arterial pressure, and electrocardiogram.

In Table 26 are shown the results of 3 experiments in which pigs received intravenous infusions of isotonic potassium chloride. The changes in the plasma concentration of the plasma and the erythrocytes are indicated in relation to changes in hematocrit, arterial pressure, and electrocardiogram.

Arterial blood pressure. The immediate effect of immersion in water of 60–75 C upon the mean arterial pressure of pigs was a rise which sometimes amounted to as much as 140 mm Hg. This rise also occurred in curarized animals or when hot water was

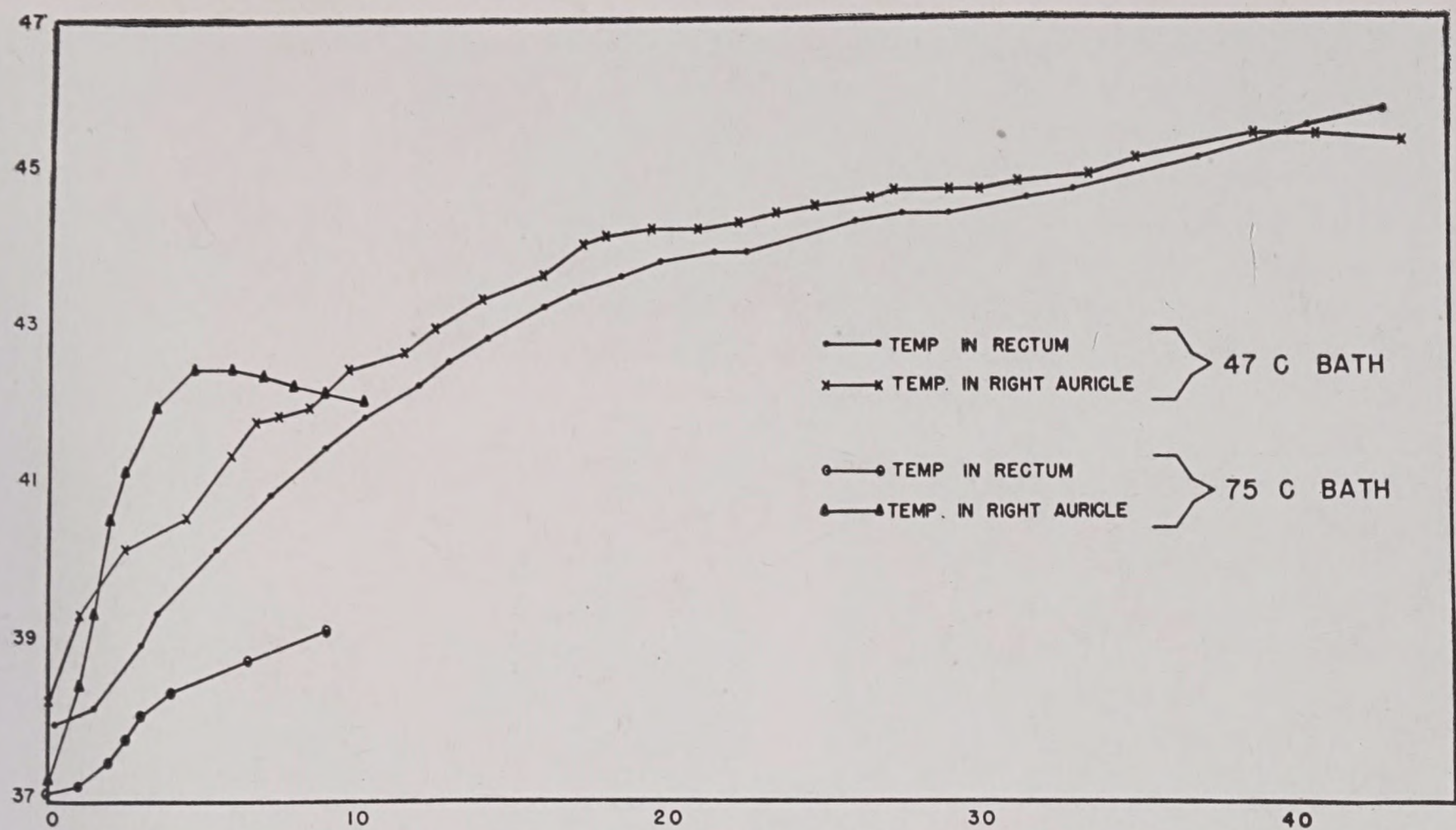


FIGURE 34. Plot of thermocouple recordings showing rate of change in rectal and right auricular blood temperatures during immersion in low (47 C) and high (75 C) temperature water baths.

47 C Pig 882 (13.2 kg) 75 C Pig 907 (10.5 kg)

It may be seen that, although right auricular blood temperature rises rapidly after immersion, there is considerable lag in temperature rise in rectum. The higher the temperature of the bath, the greater is the difference between the two.

splashed on the skin. It was absent at immersion temperatures of 45–47 C.

At temperatures of 44–59 C the blood pressure was maintained at or above preimmersion level for 16 to 26 minutes. It began to fall at variable times during exposure, and reached half of the original value in 17.5–41 minutes. The rectal temperature at this time had risen from 34.3–40.1 C to 42–44 C. These animals died after 25.5 to 50 minutes with rectal temperatures of 43.9–45.8 C, the heavier pigs surviving somewhat longer than the lighter ones. Heart temperatures were within a few tenths of a degree of these values (Figure 34).

In pigs exposed to water of 60–75 C, the arterial pressure was maintained for 1–6 minutes, and reached half of its original value in 5.5–11 minutes. The animals died after 8–15 minutes with rectal temperatures varying from 39.4–46.0 C. However, the discrepancy between heart and rectal temperature often was considerable (Figure 34).

The possible reversibility of the fall in arterial pressure was investigated. Immersion of a pig at 47 C for 33 minutes produced a fall in blood pressure from 104 to 40 mm Hg (Figure 35). Exposure to cool water brought the pressure back to its original level and lowered the rectal temperature from 43.3 to 42.0 C. Re-exposure to 47 C again resulted in a fall in blood pressure, and death occurred at a rectal

TABLE 25. Rectal temperature, arterial pressure, electrocardiogram, hematocrit, and hemoglobin and potassium content of plasma and of red blood cells of 5 dogs immersed in hot water.

A — Normal sinus rhythm (normal rate, tachycardia or bradycardia). Normal duration of QRS complex.
B — Slight widening of QRS complex without P wave.

Time	Rectal	Arterial		K plasma
min sec	temp C	pressure mm Hg	ECG	milliequiv/l
Dog 931 (7.4 kg) 55 C. Died after 23 min.				
Control	35.4	112	A	2.8
5 10	37.0	92	A	5.2
13 15	40.6	58	A	4.7
20 45	41.4	18	A	6.9
Dog 930 (7.5 kg) 60 C. Died after 16.5 min.				
Control	36.9	100	A	4.0
4 45	37.4	86	A	3.3
7 55	38.0	64	A	4.7
10 40	39.1	66	A	5.3
Dog 922 (8.5 kg) 75 C. Died after 15 min.				
Control	37.9	118	A	3.1
2 55	37.6	90	A	5.8
6 30	38.4	68	A	6.4
10 20	39.0	76	A	5.8
15	39.3	30	A	6.8
Dog 929 (8.2 kg) 75 C. Died after 13.5 min.				
Control	37.2	130	A	3.9
3 10	38.5	130	A	4.8
8 30	42.1	120	A	6.1
12 45	44.1	74	B	8.2
Dog 934 (7.6 kg) 75 C. Died after 25 min.				
Control	34.6*	148	A	3.1
15 16	41.7*	100	A	..
24 45	43.5*	72	A	6.9

* Right heart temperature.

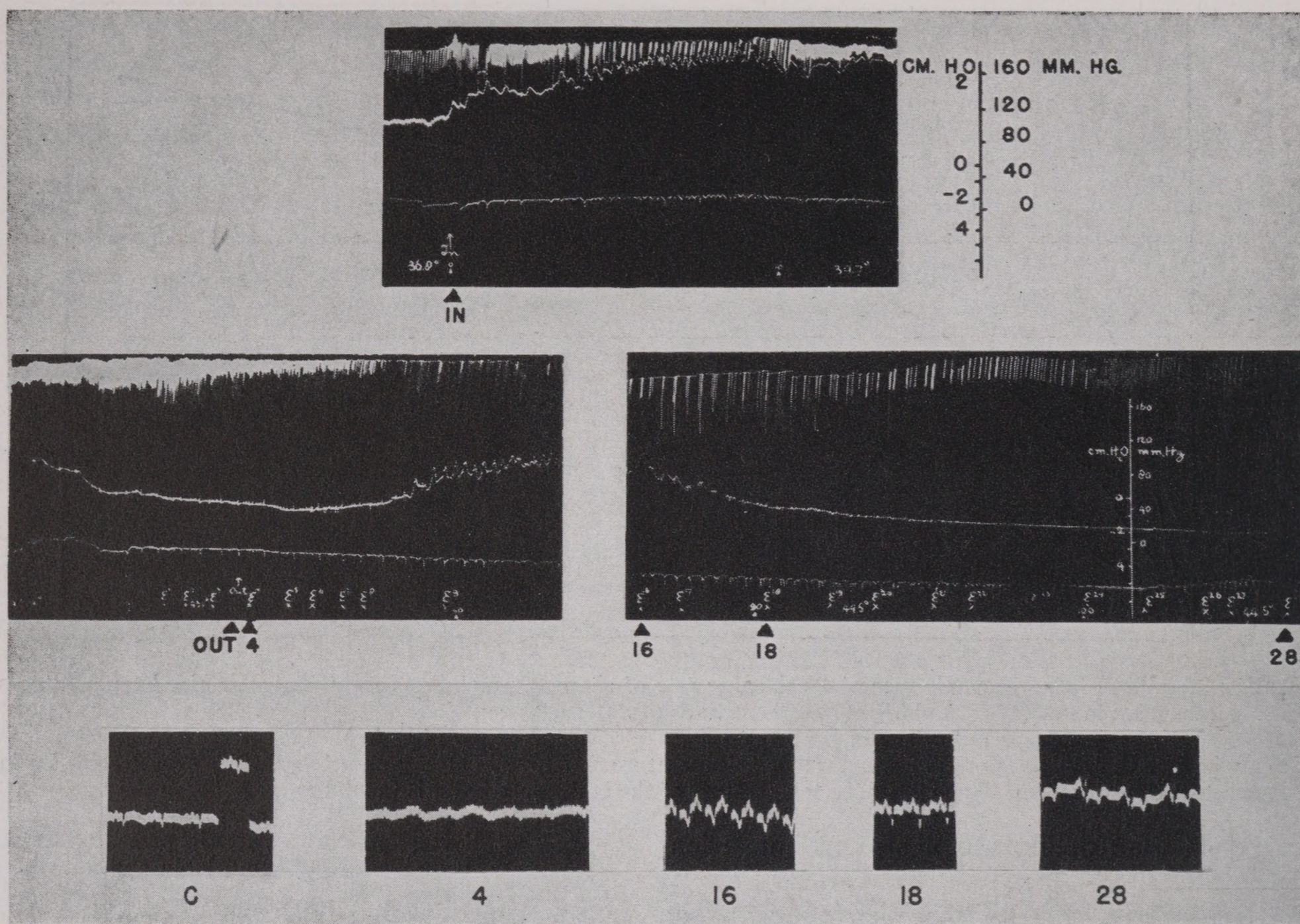


FIGURE 35. Effect of two episodes of cutaneous hyperthermia on pig 879 (11.8 kg) caused by immersion in water at 47 C. First period of immersion lasted for 33.5 minutes and is indicated by words "in" and "out" on first and second segments of kymograph record. Fifteen minutes after end of first period of hot water immersion and between second and third segments of record, animal was immersed again at 47 C and allowed to remain in bath until dead (56.5 minutes). Between two episodes of hot water immersion, skin temperature was lowered by exposure to cool water. Total duration of experiment was 105 minutes. Upper, middle, and lower tracings on the kymograph record represent respectively pneumogram, carotid pressure, and right auricular pressure. The numbers under the electrocardiograms correspond to those under the kymograph tracings; C = control period.

temperature of 44.5 C. In another instance exposure to water of 75 C for 1 minute reduced the pressure from 140 to 20 mm Hg in 5 minutes. During subsequent exposure to room air the pressure recovered, and reached 130 mm Hg after 73 minutes. The animal was still alive after more than 2 hours. Exposure of one animal to water of 75 C for 5 minutes resulted in a fall in blood pressure from 138 to 78 immediately after immersion. The pressure continued to fall, and the animal died after 18 minutes.

The arterial pressure in dogs behaved in a way comparable with that in pigs at the same temperature. Animals immersed at 60–75 C survived for 13.5–25 minutes.

Right auricular pressure: Intra-auricular pressures of pigs before immersion varied from +32 to -66 mm H₂O (average -23 mm H₂O). In only three

out of fifteen animals was the pressure in the right auricle higher than atmospheric (+13, 20, and 32 mm H₂O). In most instances, a slight rise occurred following immersion, the control level being regained in 0.5 to 3 minutes. In five of the six animals immersed at 44–49 C, this was followed by a gradual drop of 4–20 mm H₂O. There was no rise in venous pressure until 1 or 2 minutes before death. In the sixth pig, immersion did not influence the auricular pressure (Figure 35).

In seven of the nine pigs exposed to water of 60–75 C, a gradual rise of the right auricular pressure was observed, beginning in the middle of or even early in exposure and continuing until death. This rise amounted to 15–45 mm H₂O and occurred at a time when both arterial pressure and respiration were still adequate (Figure 36). In some instances, it

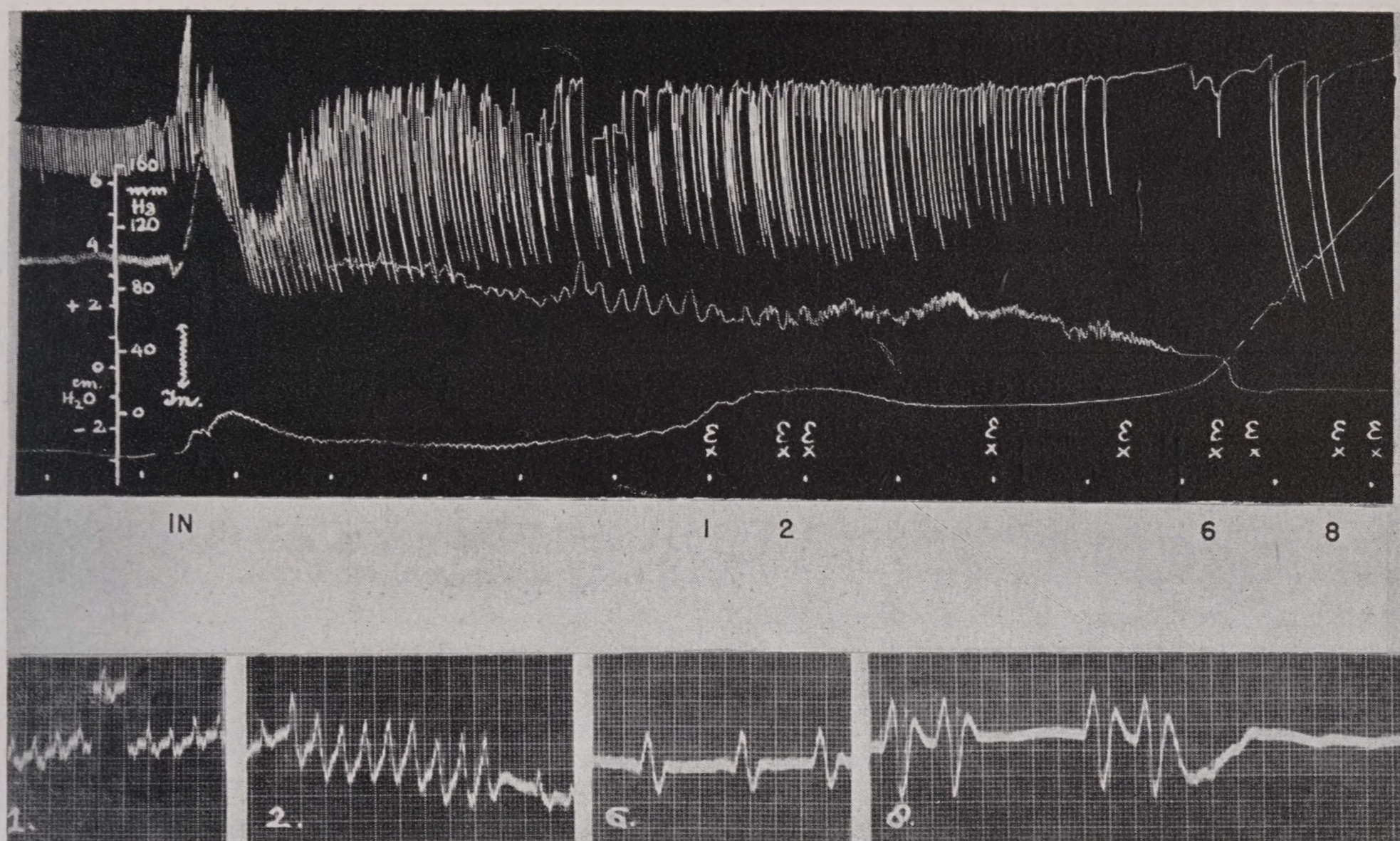


FIGURE 36. Effect on pig 871 (9.1 kg) of immersion in water bath at 70–73 C for 12 minutes. Upper, middle, and lower tracings on kymograph record represent respectively pneumogram, carotid pressure, and right auricular pressure. Sequence in which electrocardiograms were taken is indicated.

was preceded by a fall of 20–30 mm H₂O which rapidly developed 1–3 minutes after the exposure had started. In two animals, this fall was the only change in auricular pressure that was observed until 1 minute before death, when it rapidly rose.

One pig, exposed for only 1 minute to water of 75 C, showed an abrupt fall of 40 mm H₂O. During the following 70 minutes the auricular pressure gradually returned to the preimmersion level, coincidentally with recovery of the arterial pressure.

The auricular pressure of four dogs was lower than that of the pigs. It ranged from –77 to –108 mm H₂O. Because of hydrostatic effects the auricular pressures before and during immersion could not be compared. However, neither in the two dogs exposed to 75 C nor in those exposed to 55 and 60 C was there observed any change in the recorded auricular pressure during the period of immersion.

Because of the possible contributions of the type or rate of breathing to the observed pressure changes, some experiments were performed on curarized pigs. Artificial respiration was applied throughout the experiments. The course of the auricular pressure was found to be identical with that of the spontaneously breathing animals. At 47–49 C a slow and moderate

fall was observed; exposure at 75 C resulted in a rise, beginning early during exposure.

Respiration: In agreement with earlier writers it was found that a rise in body temperature was associated with a pronounced increase in respiratory rate. In the pig the immediate effect of immersion was usually a short period of very deep and fairly rapid respirations, followed by a variable episode of only moderately increased breathing (rate 20–40). In the animals exposed to the lower temperature range the onset of respiratory rates of 170–200 was often sudden, and occurred in the first 10 minutes of exposure, at rectal temperatures of 39–41 C. Deep gasps interrupted this shallow tachypnea. The arterial blood maintained its bright red color. The tachypnea gradually increased, and rates of 300 were not infrequently reached. When the rectal temperature had mounted to 43–44 C, breathing abruptly slowed to 10–40 per minute and became much deeper. Additional slowing usually continued until death. In the dog, immersion was immediately followed by a tachypnea of 100–150 per minute, which gradually increased. Rates over 200 were not encountered.

It is difficult to estimate whether the respiratory

TABLE 26. Physiological and chemical changes in three pigs intravenously infused with an isotonic (1.12%) solution of KCl.

A — Normal sinus rhythm (normal rate, tachycardia or bradycardia).
Normal duration of *QRS* complex.

B — Slight
BB — Moderate
BBB — Pronounced

Widening of *QRS* complex without *P* wave.

BBB — Can often be interpreted as ventricular fibrillation.

	Time		Arterial pressure			K plasma	K cells
	min	sec	mm Hg	ECG	Hematocrit	milliequiv/l	milliequiv/l
Pig 901 (14.8 kg). Rate of infusion 0.6 cc/kg/min. Died after 50 min.							
Control	..	..	..	A* (lead I)	36	4.3	123
11	00	..	..	A*	36	9.0	125
16	00	..	..	A*	37	9.5	124
18	00	..	..	BB	38	11.2	121
26	00	..	..	BBB	37	15.5	132
Infusion stopped							
26	10	..	..	O	..	...	...
35	00	..	..	O	..	...	...
36	00	..	..	A	..	...	...
41	00	..	..	A	38	8.7	139
41	30	Infusion started again. Rate 0.7 cc/kg/min.					
50	00	..	..	BBB	35	17.7	136
Pig 911 (8.7 kg). Rate of infusion 0.9 cc/kg/min. Died after 22.5 min.							
Control	..	..	..	A (lead II)	35	3.2	127
11	00	..	..	A†	34	8.7	122
14	00	..	..	B	35	10.6	122
16	00	..	..	BBB	35	12.7	125
20	00	..	..	BBB	31	27.0	...
22	00	..	..	O	28	38.0	127
Pig 925 (15.9 kg). Rate of infusion 0.6 cc/kg/min. Died after 39 min.							
Control	..		76	A (lead II)	33	3.5	112
6	08		76	A	33	5.7	117
12	40		76	A	32	10.6	114
19	37		76	A‡	34	12.7	110
24	50		76	B	37	15.7	109
35	18		24	BBB	37	26.1	111

* *P* wave not clearly shown.

† *P* wave getting blunt.

‡ *P* wave very flat.

or the circulatory system failed first in these animals. If bradypnea is considered as the first manifestation of failing respiration it might be said that the cardiovascular system survived somewhat longer, as judged by the presence of an appreciable arterial blood pressure. However, at least in the beginning of bradypnea, the pulmonary ventilation certainly was as adequate as during the control period. If the onset of prolonged apnea is considered as the end point of adequate respiratory function, both systems failed simultaneously. In three animals, artificial respiration was applied at a time when the arterial pressure was still appreciable (80–90 mm Hg), without having the slightest effect upon its downward course. Moreover, the final rectal and heart temperatures of the curarized pigs fell well within the range of those of spontaneously breathing animals.

Exposure of pigs to 60–75 C produced an increase in respiratory rate which did not exceed 80–90 per minute. The breathing remained deep until the terminal episode of bradypnea, ending in occasional deep gasps. In dogs the respiratory changes were essentially the same as those encountered at the lower temperatures.

Electrocardiographic changes: In both pigs and dogs, the first change, beginning immediately after immersion, consisted of a progressive increase in heart rate to levels of 300–350 per minute. Associated with this increase, changes occurred in the *QRS* complex, consisting of decrease in amplitude of the *R* wave and deepening of the *S* wave or vice versa with maintenance of the normal *QRS* interval; and inversion of the *T* wave. The changes in the initial ventricular deflection might in part at least be due to

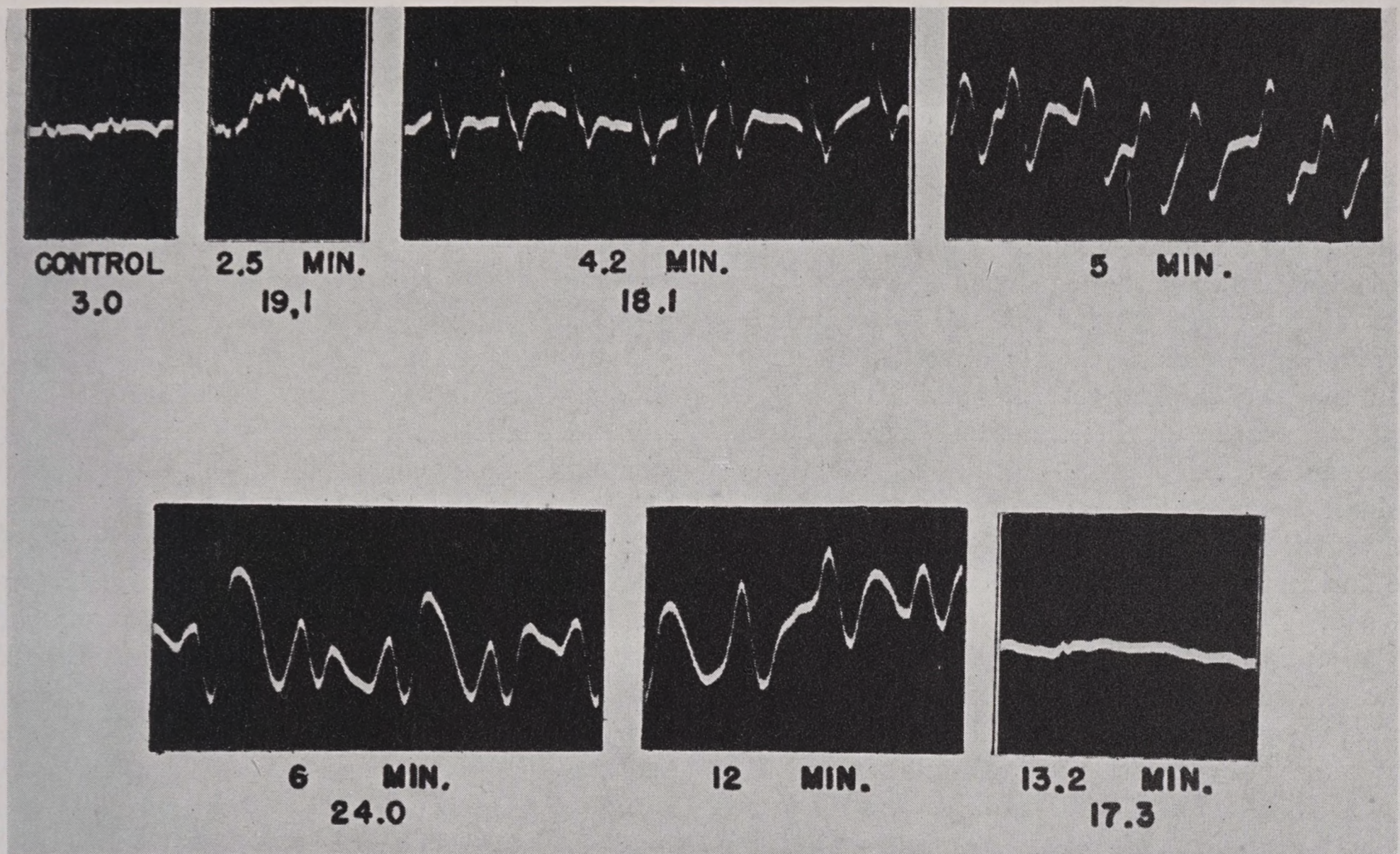


FIGURE 37. Relationship between plasma potassium level and changes in electrocardiogram (lead 1) during immersion of pig 910 (9.5 kg) in water bath at 72–75°C. Plasma potassium values are given in milliequiv/l. Death occurred 12.5 minutes after beginning of experiment.

variations in type of breathing with resulting changes in the position of the heart. (Harris.²³) They occurred only to a minor degree in curarized animals.

In the pig, the abnormalities following this sinus tachycardia varied markedly with the temperature of exposure. Of all animals exposed to water at 44–50°C (Tables 23 and 24) only one showed appreciable widening of the *QRS* complex and loss of *P* wave. This occurred 1 minute before death. Another animal showed disappearance of the *P* waves.

The changes in the remaining pigs were limited to sinus bradycardia and sinus arrhythmia, which became most pronounced 2 or 3 minutes before death (Figure 35). Occasionally, auriculoventricular block of varying degree was seen during this period.

In contrast, eleven pigs continuously exposed to temperatures of 64–75°C (Tables 23 and 24) all showed the gradual development of exceedingly wide ventricular complexes with very large *T* waves, and the gradual disappearance of the *P* wave.^k The gen-

eral shape of these complexes resembled that of the original supraventricular ones. Their development was usually associated with definite slowing, although the heart rate remained regular. In some cases, the transitional phase consisted of salvos of fairly rapid and wide ventricular complexes, which interrupted a still-existent sinus bradycardia. In the terminal stage, the initial ventricular deflection could not be separated from the final one. The electrocardiogram consisted either of very slow, extremely wide ventricular waves, separated from each other by isoelectric intervals of 0.2–1.0 second, or of more rapid variations at 160–240 per minute, in which one wave merged with the next. The latter state might be called ventricular fibrillation (see Figures 36 and 37).

In nine of the eleven pigs, these changes made their first appearance early during immersion, at rectal temperatures of 37.0 to 41.6°C and at a time when the arterial pressure and respiration were still adequate. In four of these, the blood pressure at the

^k During tachycardia, actual observation of this disappearance was impossible because of overlapping of *P* and preceding *T* waves. In these instances, it was assumed that the same changes had taken place as in the instances where

the *P* wave could be followed through a stage of decreasing amplitude to disappearance, as subsequent slowing of the beat similarly revealed the absence of auricular complexes.

time of onset of the wide complexes was actually equal to or higher than that before immersion. In only two animals were the abnormalities first noticed when the pressure had fallen to low levels, and it is possible that they would have been demonstrated earlier if more electrocardiograms had been taken. Exposure for 6.5 and 5 minutes similarly resulted in marked widening of the *QRS* complex, whereas exposure for 3 minutes and 1 minute did not produce deviations other than those at lower temperatures.

In the dog, the electrocardiographic changes at high temperatures were in no way different from those encountered at 44–50 C (Table 25). They were limited to an increase in rate and to minor changes in the ventricular complex. No widening occurred and the auricular manifestations remained present until the end.

Chemical Changes. For a complete discussion of the effect of temperature on the potassium concentration of the plasma, see Section 17.10 of this chapter. The potassium concentration of the plasma of fifteen pigs in which physiological studies were made are shown in Table 24. The initial plasma levels ranged between 3.0 and 4.8 milliequiv/l. The potassium concentration of the red blood cells ranged from 113 to 145 milliequiv/l. The course of these concentrations during immersion varied markedly with the temperature.

Immersion of four pigs at 47 C produced a gradual and sustained rise in plasma potassium. Ten minutes exposure resulted in levels of about 6.0 milliequiv/l. During the rest of the exposure, the level increased by an additional 1 to 4 milliequiv. The highest level was 10.2 milliequiv/l obtained 30 seconds before death.

On the other hand, continuous exposure at 70–75 C characteristically resulted in an enormous rise in the plasma potassium level. This increase was found to take place with surprising rapidity. In five pigs, the plasma after 1 to 4 minutes of exposure contained 14.2 to 25.5 milliequiv/l of potassium. A sample drawn in this period from one curarized pig was still essentially normal and the peak observed in this animal was only 11.9 milliequiv. Peaks from 16.7 to 25.5 milliequiv were observed in six pigs during exposure. Curare did not prevent rises in this range in two pigs; however, no early observations were made on these animals. In some instances, the potassium level fell toward the end. However, it remained markedly elevated.

In some experiments, the exposure was terminated

before the animal had expired. Immersion for 6.5 and 5 minutes similarly resulted in a tremendous rise of plasma potassium. At the time of death, the level was still very high. Immersion for 3- and 1-minute periods produced a less pronounced increase; at the time of death, the level was only 2–2.5 times the normal one.

17.11.4

Discussion

These observations show that the physiological disturbances leading to death in pigs exposed to water at 46–50 C are of a different nature from those encountered in animals exposed to temperatures of 60–75 C.

In pigs immersed at the lower temperatures, the occurrence of a gradual fall in right auricular pressure followed by a fall in mean arterial pressure indicates a progressive decrease in venous return to the heart. That this decrease, at least during a major part of the exposure, was due to an increase in capacity of the peripheral vascular bed, rather than to loss of intravascular fluid, is evident from the fact that the changes in circulatory dynamics were found to be reversible to a considerable degree. As the exposure continued, the detrimental effects of the heated blood upon the heart muscle were added to the peripheral effects, and both factors undoubtedly contributed to the lethal ending.

It is difficult to say whether cardiovascular failure or respiratory insufficiency was the immediate cause of death. Profound arterial hypotension and pronounced bradypnea were usually encountered at the same time. It can be said, however, that the mean arterial pressure fell considerably before any impairment in respiratory function was evident. Artificial respiration applied at a time when the arterial pressure was still appreciable had no effect upon its downward course. Moreover, curarized pigs did not survive longer than spontaneously breathing animals; all but one animal died after 25 to 51 minutes of continuous immersion. The plasma potassium level increased by 66–250 per cent; the highest level found was 10.2 milliequiv/l. No profound changes in cardiac function, as judged by the electrocardiogram, occurred. As will be shown, plasma potassium levels up to 10 milliequiv/l do not produce significant changes in intraventricular conduction.

At immersion temperatures of 60–75 C, the pigs survived for only 8 to 15 minutes. In the middle of the exposure, or even earlier, at a time when the respiration was still adequate and the mean arterial

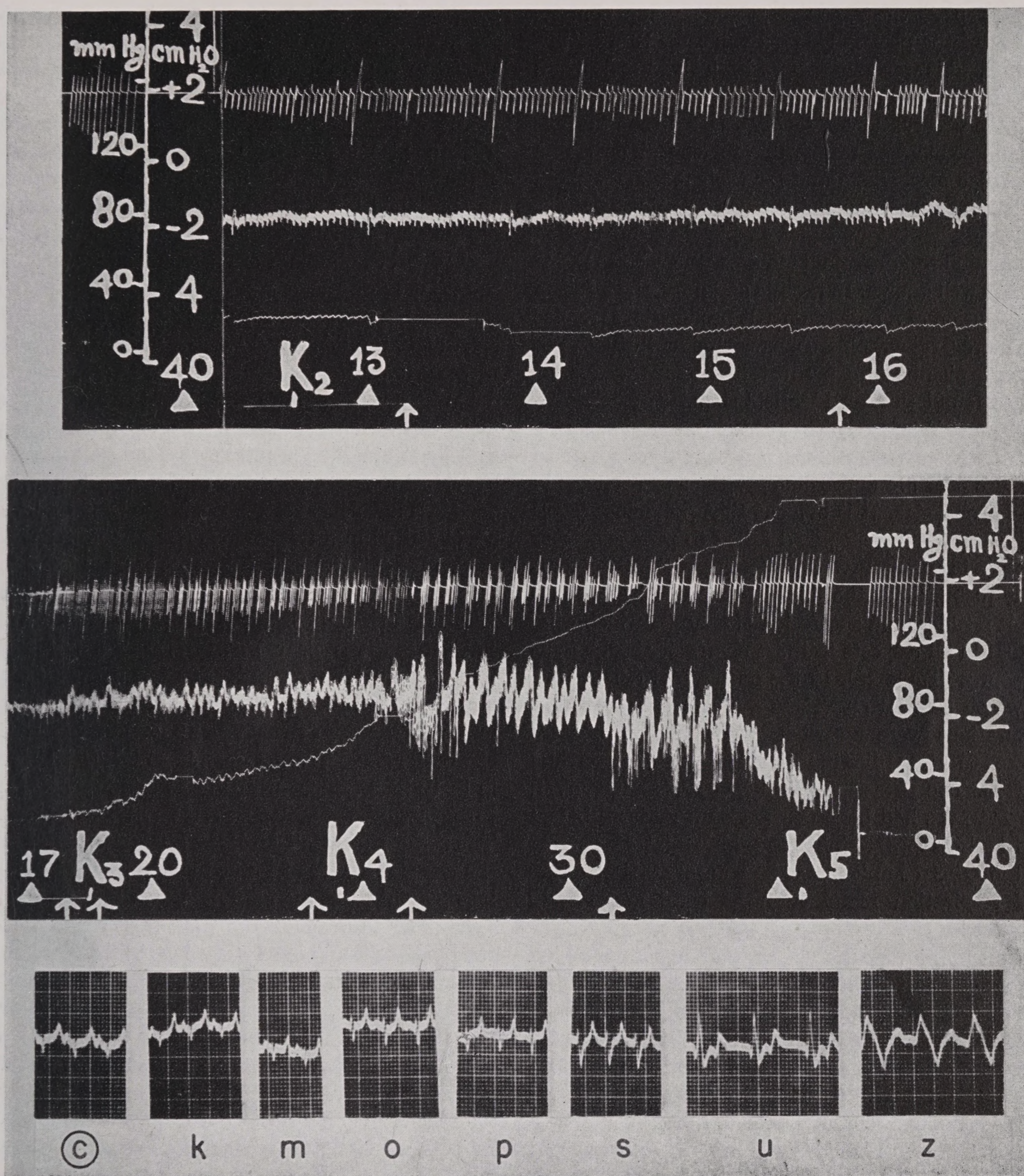


FIGURE 38. Effect of continuous intravenous infusion of 1.12 percent KCl at the rate of 0.6 kg/min. Upper, middle, and lower tracings on kymograph record represent respectively pneumogram, carotid pressure, and right auricular pressure. Time in minutes is shown at base of record. Time at which blood samples were taken is indicated by symbols K₂, K₃, K₄, and K₅. The times at which the sequence of electrocardiograms (k to z) were taken are indicated by arrows. See pig 925, Table 26, for corresponding potassium levels.

pressure was still considerable, pronounced changes in cardiovascular function made their appearance. They consisted of a rise in right auricular pressure,

and electrocardiographic changes in the form of disappearance of the *P* wave and progressive widening of the *QRS* complex, often terminating in ventricular

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fibrillation. At the same time, the potassium concentration of the plasma reached values of 16–19 milliequiv/l. This was associated with a striking destruction of red blood cells.

These observations strongly suggest that the hyperpotassemia was responsible for the disturbances in cardiac mechanism and for the subsequent myocardial failure evidenced by the rise in auricular pressure. That the damaging effects of a rising plasma potassium level first of all manifest themselves in the heart is well known. In rabbits and dogs, the infusion of a solution of a potassium salt produces a sequence of electrocardiographic changes similar to those observed in pigs during exposure to high temperatures.^{38,48} It was found that an identical sequence of changes takes place in infused pigs (Table 26). In two animals, infusion rates were maintained that were likely to produce death in approximately the same time as in the burned pigs. It is evident that potassium levels of less than 10 milliequiv/l failed to produce either changes in the *P* wave or widening of the *QRS* complex, just as was the case in burned pigs. Higher levels resulted in a succession of changes which were similar in all respects to those observed at high temperatures (Table 24). In the one animal (Figure 38) in which arterial and right auricular pressure and respirations were recorded, the auricular pressure began to rise 19 minutes after the infusion had started. The potassium level was 12.7 milliequiv/l; the *P* waves had begun to flatten 3 minutes before and had disappeared. Three minutes later widening of the *QRS* complex began. The arterial pressure and respiration remained normal for another 10 minutes.¹

That the cardiac changes due to the potassium ion are reversible to a remarkable degree is clear from experiment 901 (Table 26). The usual succession of electrocardiographic changes was observed until, some seconds after a potassium level of 15.5 milliequiv/l had been reached, the string shadow remained resting. The infusion was stopped. No electric or auscultatory evidence of cardiac activity could be demonstrated for the following 10 minutes, although the animal continued to breathe at a very slow rate. Then heart action returned and respiration became more rapid. The electrocardiogram had returned to normal. A plasma sample taken 5 min-

utes thereafter contained 8.7 milliequiv/l of potassium. Infusion was started again, the well-known changes were again observed, and the pig died with a potassium level of 17.7 milliequiv/l.

The rapidity with which potassium is removed from the plasma makes it imperative that the release of the ion into the circulation be intensive enough and be continued for a sufficiently long time to lead to death. This actually occurs in the burned pigs. The liberation of potassium often occurred at so rapid a rate that there was a lag between the rise in potassium and the electric changes. Thus, in pig 910 a level of 19.0 milliequiv/l was reached in 2 minutes, whereas more than 4 minutes were required to produce the typical widening. Animals exposed to high temperatures for only 1 or 3 minutes did not release sufficient potassium to produce a characteristic effect on the heart, whereas exposure for 6.5 minutes was adequate in this respect. Exposure to 75 C for 5 minutes resulted in a tremendous rise in potassium and in electrocardiographic changes, but even here both manifestations diminished in intensity during the following 14 minutes.

Although it is clear that in pigs exposed to high (60 to 75 C) temperatures the most striking physiological disturbances are those which result from the release of excessive amounts of potassium, continued exposure results in a progressive and generalized rise in body temperature which undoubtedly causes disturbances other than those due to hyperpotassemia. Thus, the peripheral and central factors that were the cause of death at lower temperatures also come into play at these high temperatures.

In order to evaluate the relative contributions of red blood cells and fixed body cells to the increase in plasma, potassium experiments were performed on dogs (Table 25). Whereas the potassium concentration of their fixed cells is similar to that of the pig, their red cells contain only small amounts. Immersion at 75 C resulted in an intense hemolysis, but the potassium level did not rise above that encountered in pigs at 47 C and electrocardiographic changes characteristic of hyperpotassemia were not seen.

The distribution of the potassium in human blood is similar to that in pig's blood, the potassium concentration of the red cells being approximately 110 milliequiv/l, that of the plasma approximately 4–5 milliequiv/l.^{32,41} High plasma potassium levels should therefore be expected in human beings in whom a major part of the body surface has been exposed to high environmental temperatures. Several

¹ The rate of infusion was slow enough so that the rise in venous pressure could not be ascribed to the administration of the isotonic salt solution *per se*.⁵

minutes of exposure would probably be required to result in the very high levels encountered in these experiments. It is also probable that, if the immediate effects of the exposure were survived, a markedly elevated plasma potassium occurring immediately following the injury would fall within the next hour. It should be remembered, of course, that a rise in plasma potassium is a normal post-mortem phenomenon.

17.11.5

Summary

There are two principal mechanisms by which exposure of the surface of the body to excessive heat may cause rapid circulatory failure and death.

In one, the systemic hyperthermia due to conduction of heat to the interior of the body by way of the blood stream leads to a rapid and progressive decline in blood pressure and failure of circulation due to peripheral vascular collapse.

In the other, the circulatory failure is principally central and is due to the effect on the heart of an excessively high concentration of potassium in the plasma. Central circulatory failure is likely to occur when the overheating of the skin and subcutaneous tissue is so intense, prolonged, and generalized that potassium is released from the erythrocytes with

such rapidity and in such large amounts as to maintain plasma levels in excess of 11 milliequiv/l.

In the case of thermal exposures of low intensity, peripheral circulatory failure may occur without sufficient rise in tissue (and blood) temperature to cause a functionally significant rise in plasma potassium. When a thermal exposure has been of sufficient severity to cause fatal hyperpotassemia, the central circulatory effects are likely to be complicated by peripheral vascular collapse.

It is essential to the development of acute hyperthermic potassium poisoning that the erythrocytes have a high original concentration of this element. Thus, fatal hyperpotassemia, due to hyperthermia, occurs in the pig but not in the dog. Since man and pig have similar potassium concentrations in erythrocytes, it is inferred that they are probably similarly susceptible to the development of fatal hyperpotassemia following cutaneous exposures to excessive heat.

Although thermally induced respiratory disturbances undoubtedly contribute to either type of circulatory failure, maintenance of pulmonary ventilation by artificial respiration does not prevent death or cause significant prolongation of the survival period.

Chapter 18

MISCELLANEOUS TOXICOLOGICAL STUDIES

By *Birdsey Renshaw*

18.1 INTRODUCTION

DIVISION 9 HAS CARRIED OUT, in its laboratories operated for toxicological and immunological studies on chemical warfare agents, a limited number of investigations with materials which were not considered for use as war gases but whose toxicological properties were for other reasons of interest to the Army, Navy, or other National Defense Research Committee [NDRC] divisions. In this chapter are summarized the results of four such investigations: (1) the pathological changes caused by prolonged exposures to oil screening smokes, (2) the toxic effects of gasoline fumes, (3) the toxicity of Salcomine dusts, and (4) the hypersensitivity and dermatitis caused by hexanitrodiphenylamine and enemy explosives containing it.

18.2 TOXICITY OF OIL SCREENING SMOKE

With the development by NDRC Division 10 of generators for the production of oil screening smokes, the question arose whether personnel exposed for prolonged periods in such smoke clouds (consisting of fine droplets of unburned hydrocarbon oils) would be subjected to health hazards. Although no information was available concerning the toxicity of oil clouds for animals or man, there were on record nearly 200 cases of "lipid pneumonia" attributed to aspiration of mineral oil.⁹ Inasmuch as lipid pneumonia may occur whenever an exogenous oil reaches the pulmonary tissues and remains for a sufficient time to cause irritation, the possibility existed that this potentially debilitating condition might result from the inhalation of the screening smokes. At the request of the Chemical Warfare Service, an experiment was performed in which mice were exposed for prolonged periods to clouds of atomized lubricating oil;¹ the continuing interest of the Service led to the extension of the tests⁴ to include the exposure of monkeys to clouds both of lubricating oil and of fog oil standardized for use in the Langmuir-type generator. The results of these tests with animals afforded no basis for supposing that prolonged exposures of military personnel to oil screening smokes in the field

would be dangerous. By now the actual use of oil screening smokes in military operations has been extensive and no evidence has been forthcoming that health hazards are involved.

The experiments were performed with animals kept for 100 days in a large closed chamber into which for 30 minutes of every hour air containing oil fog was passed at a rate of 0.8 chamber volume per minute. In the experiments with lubricating oil (Penn Oil, SAE No. 10), the nominal concentration was 132 $\mu\text{g}/\text{l}$ and the droplets varied in diameter from about 0.3–1.5 μ ; the mass median diameter was 1.4 μ . In the case of fog oil (Texas Company, SGF No. 1 Oil) the analytical concentration was 65 $\mu\text{g}/\text{l}$.

The death rate among the mice exposed to the clouds of atomized lubricating oil was not significantly different from that in the normal colony and the animals showed no serious pathological changes during or at the end of the exposure.¹ No free oil was ever seen in the alveoli or bronchi, and chemical analyses at the end of the 100 days revealed that relatively little had accumulated, there being in the lungs 1.65 mg per mouse (0.4 per cent of the total lung weight). Occasional oil-containing macrophages could be seen after the experiment had been in progress for a week. These increased in number during the first 35 days, after which time almost every alveolus contained at least one such cell, but they did not become significantly more numerous during the subsequent two-thirds of the exposure period. The tracheo-bronchial lymph nodes of mice sacrificed after 3 weeks in the chamber showed accumulations of oil-containing macrophages, but there was no reaction to them.

These essentially negative results with mice led to repetition of the experiments with the Rhesus monkey — a species which, in terms of posture and size of respiratory passages, more closely resembles man.⁴ Chemical analyses of the lungs of exposed animals revealed a progressive accumulation of oil to a maximum of about 10 per cent of the dry weight, or 2 per cent of the wet weight, at the end of the 100 days; approximately one-half of this accumulation had disappeared a year after the start of the exposure. Microscopic examination revealed some free oil, and

oil-laden macrophages were scattered throughout the lung, in the alveoli, subpleurally, and in the bronchial and pleural lymphatics. However, little inflammatory reaction attributable to the oil occurred, and subsequent to the exposure the fibroplastic reaction to the remaining oil was slight. The one conspicuous extrapulmonary effect was loss of hair during the prolonged exposure, and its subsequent regrowth.

In so far as the animal findings may be applied to man, the failure of large amounts of oil to accumulate and the absence of severe acute and chronic reactions make it improbable that significant pulmonary effects would be produced by any exposures likely to be encountered.

The results of exposure to fog oil (SGF No. 1) were similar, with one important exception. Six of seven monkeys died, apparently of starvation, during or shortly after the termination of the exposure. Examination of the stomachs revealed acute or hypertrophic gastritis and, in those dying after the greatest delays, the picture of an adenoma malignum superimposed on hyperplastic gastritis. These serious pathological changes are believed to have been induced by carcinogenic agents present in ingested oil; carcinogens have been found in petroleum oils¹⁴ and presumptive evidence compatible with their presence in SGF No. 1 oil was obtained. Thus, the possibility exists that cancer might result from prolonged exposure to oil smokes. However, it should be noted that the monkeys, their surroundings, and their food were continually covered with oil, and the animals therefore undoubtedly ingested much oil in addition to that which they breathed and swallowed.

18.3 TOXICITY OF GASOLINE FUMES

Early in 1944 reports were received that among the individuals killed in flame thrower attacks upon enclosed fortifications were some who had not sustained severe burns. The Chemical Warfare Service was interested in determining the cause of these deaths and, as a small part of a larger program, requested that the effects of short exposures to the vapors from unburned flame thrower fuel be determined.

As it did not prove feasible to set up toxic concentrations of vapor from thickened flame thrower fuel, the tests^{5a} were limited to experiments with unthickened gasoline compounded to meet Federal Specification VVM-564. The gasoline was atomized into an airstream which was heated to vaporize the

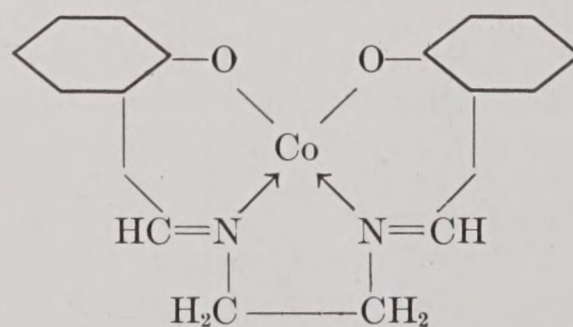
droplets before passing to an animal chamber in which the air temperature was about 35 C. The $L(Ct)_{50}$'s for 5-minute exposures of mice, rats, and guinea pigs were very high, in the order of 300 mg/l ($Ct = 1,500,000$ mg min/m³, analytical). This concentration was somewhat above the saturation value at the temperature of the chamber, and a dense cloud formed. The mice and rats surviving the exposure period remained narcotized for 10 minutes but appeared normal within one-half hour; no gross pathological changes were produced. The guinea pigs exhibited rapid, shallow breathing with forced inspirations; autopsies revealed bronchospasm and emphysema. It is probable that the action of the gasoline fumes on mice and rats was purely narcotic, and that in guinea pigs this action was augmented by bronchospasm.

The above-mentioned concentration is above the upper explosive limit for gasoline and could not be built up in the presence of flame. There is no doubt, on the other hand, that concentrations of gasoline vapor rapidly lethal for man as well as animals can be attained in closed spaces in the absence of flame,¹⁰ and there is no information as to whether or not sensitivity to the vapors is markedly enhanced at greatly elevated ambient temperatures.

18.4 TOXICITY OF SALCOMINE DUSTS

Early in 1942 the success of NDRC Division 11 in developing Salcomine^a oxygen generators for use on shipboard and elsewhere led to the need for an investigation of the possible industrial hazards which might be involved in manufacturing and working with this compound.

^a Salicylaldehyde ethylenediimine cobalt, known as Salcomine, has the following structure:



This material has the property of absorbing oxygen (about 4 per cent by weight) when exposed to air, and of releasing the absorbed oxygen when heated. Since this cycle may be repeated many times, it is possible to construct systems employing Salcomine for the separation of atmospheric oxygen. For details the reader is referred to the Summary Technical Report of NDRC Division 11, Section 11.1. No doubt the Salcomine samples used in the toxicological studies were partially oxygenated.

Preliminary tests² revealed that Salcomine dust is toxic upon inhalation and clearly indicated the necessity of taking precautions to protect workers. Mice exposed for several hours to the dust at nominal concentrations of 0.4–2.4 mg/l (undoubtedly the actual, or analytical, concentrations were much lower) frequently died within 1–6 days. Autopsies revealed many pathological changes attributable to the Salcomine: there was generalized degeneration and localized necrosis of the epithelium of the trachea and principal bronchi; the lungs were hyperemic and, particularly in the peripheral portions of the lobules, edematous; the thymus gland and lymph nodes contained fragmenting lymphocytes in moderate number; and fat stains revealed fatty changes in the liver.

In 1944 the occurrence of a number of clinical cases of poisoning, presumably due to Salcomine dusts, occurred^b and prompted further animal studies.^{5b}

^b Medical examinations of eleven men⁶ exposed to small amounts of Salcomine dust revealed that the compound produced irritation of the eyes, nose, larynx, and bronchi. The symptoms, which appeared shortly after exposure and resembled those of an upper respiratory infection, cleared up after removal from exposure. Signs possibly indicative of mild systemic effects — muscular aches, nausea, and vomiting — appeared after latency of 5–24 hours in some of the subjects. In general the respiratory symptoms disappeared within a day but the digestion was sometimes upset for 3 days. There may have been some slight cumulative effect, because it was reported that chronic exposure led to anemia, lack of energy, and need for increased sleep. No permanent effects were noted and it was concluded that, with reasonable precautions including use of dust respirators, no marked industrial hazard was involved. One case with much more severe systemic effects has been reported.⁷ An emergency obliged the subject to work without a mask for a short period in an atmosphere laden with Salcomine dust. On the evening of the exposure there were no pronounced symptoms other than discomfort in breathing, but the following day abdominal pains of sufficient severity to require hospitalization and treatment with morphine developed. The subject had nausea, vomiting, and a fever. A tentative diagnosis of acute duodenitis was made and his liver became progressively more enlarged and tender. Tests performed 48 hours after admission revealed definite liver damage. The liver condition with accompanying jaundice gradually improved but the abdominal signs persisted. Penicillin was utilized. An exploratory laparotomy 2 months after exposure revealed a retroperitoneal abscess in the left lower quadrant; this was removed, as was a second similar abscess which formed on the right side 3 months later. It was suspected that other abscesses were present deep in the liver tissue, but none required drainage. Definite hardening of the liver due to scar tissue persisted. Attending physicians assumed that the inhaled and ingested dust was responsible for the acute digestive disturbances that followed exposure and led to disease of the liver, duodenum, and retroperitoneal tissues. Compare results of experimental animal exposures.

The results left no doubt that Salcomine is both a respiratory and a systemic poison and that precautions must be taken against the inhalation of its dust.

A single exposure to a high concentration killed guinea pigs immediately and mice after varying latencies. The lungs of the guinea pigs were markedly distended with air and microscopic examination revealed that the bronchi and bronchioles were strongly constricted. Mice dying soon after such an exposure exhibited no visible changes which would account for death; those dying after 1 day or more exhibited a diffuse pneumonitis, suppurative tracheobronchitis, and occasionally jaundice and coagulative necrosis of the liver.

More important from the standpoint of the health hazard and for revealing the generalized toxic effects of Salcomine are prolonged exposures to low concentrations. Accordingly, animals were exposed for 1 hour daily in a chamber to air which contained the finer particles of Salcomine dust at concentrations in the order of 100 $\mu\text{g}/\text{l}$. Three to six such exposures, corresponding to a total dosage of about 20,000 $\text{mg min}/\text{m}^3$, sufficed to kill approximately one-half of the exposed mice and rats;^c rabbits probably were not much more resistant, but guinea pigs proved to be considerably less sensitive. The mice developed a diffuse pneumonitis and tracheobronchitis, parenchymatous degeneration of the renal tubular epithelium, jaundice, and liquefying coagulative necrosis of the liver. Autopsies of rats sacrificed daily during the exposures revealed a gradually developing diffuse pneumonitis and tracheobronchitis; focal hepatitis with occasional necrosis also developed and was followed by the appearance of intracellular fat; parenchymatous degeneration of the renal tubular epithelium occurred, followed by the appearance of severe fatty changes; and in the duodenum and jejunum the epithelial cells of the mucosal glands began to show vesiculation, swelling, and many mitoses. These changes subsided after the exposures were discontinued. Clinical pathological studies on rats revealed an increase in the urinary output after the first and subsequent exposures, the development of a mucoid diarrhea, a rise in hemoglobin and red cell count, and a 5 per cent loss in body weight. A leucocytosis also developed during the exposures and subsided rapidly upon their cessation.

^c To illustrate the toxic potency of the dust it may be noted that for 6-hour exposures the $L(Ct)_{50}$ of mustard gas vapor for mice is 4,100 $\text{mg min}/\text{m}^3$, and for rats, 1,500 $\text{mg min}/\text{m}^3$.⁸

18.5 HYPERSENSITIVITY AND DERMATITIS CAUSED BY HEXANITRODIPHENYLAMINE

In 1943 the Navy Department reported the occurrence of acute dermatitis in personnel of both British and United States armed forces who had come in physical contact with enemy explosives containing hexanitrodiphenylamine, and requested NDRC to investigate the cause of the dermatitis and methods for its prevention and treatment.

A survey revealed numerous statements in the literature that hexanitrodiphenylamine is a powerful dermatitic agent, but the factual basis for this impression proved to be weak. Furthermore, dinitrochlorobenzene, a potent dermatitic and sensitizing agent for both man and the guinea pig,¹¹⁻¹³ is employed in the manufacture of hexanitrodiphenylamine, and there is the possibility that this or other intermediates or by-products may have been responsible for those cases of dermatitis which have been observed. At du Pont Company plants, which manufactured limited quantities of hexanitrodiphenylamine in 1918 and 1940, care was taken to avoid exposure to the substance and no noteworthy or severe cases of dermatitis occurred.

Inasmuch as no samples of enemy explosives known to have produced dermatitis were available, the investigation³ was confined to studies with a highly purified laboratory-prepared sample of hexanitrodiphenylamine and with a preparation from a Japanese torpedo booster. Crystallographic analysis revealed the latter to contain about 75 per cent hexanitrodiphenylamine, about 25 per cent trinitrotoluene (TNT), and no dinitrochlorobenzene; if any minor constituents were present, they totalled less than 1 per cent.

After rigorous applications of both preparations had failed to produce irritation or sensitization in guinea pigs and swine, tests were carried out on men. Neither the pure material nor the Japanese explosive proved to be a primary irritant when applied in hot weather to skin of the forearm as a saturated solution in acetone or as a powder covered by an occlusive dressing. In 2 of 29 men treated with the purified hexanitrodiphenylamine and in 1 of 31 treated with the Japanese explosive, however, "flareup" dermatitis developed about a week after the second of two applications. The dermatitis cleared up under simple symptomatic treatment within 7-10 days. Patch tests later showed that these 3 men had become markedly hypersensitive, whereas the 57 other subjects had not.

The residue from an incompletely detonated sample of hexanitrodiphenylamine was innocuous to hypersensitive skin. Prolonged treatment with excess potassium sulfide likewise rendered the explosive harmless, but treatment of acetone solutions of it with sodium hydrosulfite did not alter its ability to cause inflammation of hypersensitive skin.

Although the findings indicated that with gross contaminations of large numbers of men, instances of skin reaction of varying degree are to be expected, it was clear that hexanitrodiphenylamine is not a primary skin irritant and that it occupies a low position among the skin-sensitizing substances. Practical preventive measures were considered to be avoidance of unnecessary contact with the substance and, inasmuch as the reactions are delayed, use of an organic solvent and soap and water as soon as possible after contamination to remove the substance from the skin and from objects with which the skin can come in contact.

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